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ICDDR,B
CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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LSD
2000

Memorandum

12 March 2000

To : Dr. Rubhana Raqib
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee

Sub : Approval of protocol # 2000-005

This has reference to your memo of 10th March 2000 attaching a modified copy of your protocol # 2000-005 entitled "To study the immune responses in Bangladeshi infants randomly allocated to iron and/or zinc or a micronuttrient mix supplementation". I am pleased to inform you that the protocol is hereby approved upon your appropriate addressing of the issues raised by the Committee made its meeting held on 23rd February 2000.

Thanking you and wishing you success in running the said study.

cc: Division Director
Laboratory Sciences Division

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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

29 February 2000

To : Dr. Rubhana Raqib
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee

Sub : Protocol # 2000-005

The Ethical Review Committee in its meeting held on 23rd February 2000 considered your protocol # 2000-005 entitled "A community based, randomized controlled trial to assess the efficacy of iron and/or zinc or a micro-nutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi infants". After thorough review and discussion, the Committee made the following observations to be addressed in your protocol:

a) the title of the protocol should be changed to reflect the study objectives.

b) item # 6 of the face sheet should be appropriately circled.

c) the consent form (both Bengali and English) has not mentioned that CMI skin test and stool sample for sIgA will be collected from the subjects.

d) sample size should be specific.

e) there are inconsistencies about the amount of blood to be taken (e.g. it is mentioned at annexure I at page 3 that 3 ml of blood will be taken whereas in the consent form it has been mentioned as 4 ml).

The Committee, therefore, requests you to incorporate the above observations in the protocol and resubmit a modified copy of it for consideration of the Chair.

Thank you.

cc: Division Director
Laboratory Sciences Division

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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

To: The Chairman, Ethical Review Committee

From: Rubhana Raqib, LSD Rubhana

Date: 10 March, 2000

Subject: Resubmission of the protocol (# 2000-005)

Enclosed please find a modified copy of the proposal (Protocol # 2000-005) resubmitted for consideration by the ERC. The queries and comments have been addressed as follows:

a) As suggested, the title of the protocol has been changed to reflect the study objective. The new title is "To study the immune responses in Bangladeshi infants randomly allocated to iron and / or zinc or a micronutrient mix supplementation".

b) Item # 6 of the face sheet has been circled.

c) As correctly pointed out, the consent forms did not mention the CMI skin test and the sIgA in stool. We apologize for the unintentional mistake. This has now been included in both Bangla and English consent forms.

d) Sample size for different variables has been given in a table for sample size calculations (page 8). For making it more specific, we added two sentences in page 8 in the footnote to the table.

e) The inconsistencies about the amount of blood drawing mentioned at annexure I at page 3, line 15 has been rectified.

I sincerely hope that the modified proposal will be accepted in its present form.

Thank You.

Attachment I

APPROVED COPY

(FACE SHEET) **ETHICAL REVIEW COMMITTEE, ICDDR,B.**

Principal Investigator: Rubhana Raqib

Application No. 2000-005

Title of Study: To study the immune responses in Bangladeshi infants randomly allocated to iron and /or zinc or a micronutrient mix supplementation.

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) _____

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.

Rubhana
Principal Investigator

Trainee

Principal Investigator: Raqib, Rubhana

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: 2000-005 Date:

RRC Approval: Yes/ No Date: 30/1/2000

ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuation)
To study the immune responses in Bangladeshi infants randomly allocated to iron and / or zinc or a micronutrient mix supplementation.

2a. Name of the Principal Investigator(s) (Last, Middle, First):	**2b. Position / Title**	**2c. Qualifications**
Rubhana Raqib, LSD	Assistant Scientist	PhD

3. Name of the Division/ Branch / Program of ICDDR,B under which the study will be carried out.
Laboratory Sciences Division

4. Contact Address of the Principal Investigator
4a. Office Location: Immunology,
Laboratory Sciences Division, Mohakhali,
Dhaka-1212, Bangladesh.

4b. Fax No: +880-2-8812529
4c. E-mail: rubhana@icddrb.org
4d. Phone / Ext: +880-2-8811751-60/2404

5. Use of Human Subjects Yes [+] No [ ]

5a. Use of Live Animal Yes [ ] No [ ]

5b. If Yes, Specify Animal Species

6. Dates of Proposed Period of Support:
(Day, Month, Year - DD/MM/YY) Year 2000

7. Cost Required for the Budget Period
7a. Ist Year ($):68,800 ***2nd Year ($):*** ***3rd Year:***
7b. Direct Cost ($) *68,800* **Total Cost ($):** 86,000 (25%)

8. 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 VI Mathan
Name of the Division Director

Signature

9/3/2000
Date of Approval

9. 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 report if a grant is awarded as a result of this application.

10. Signature of PI

Rubhana

Date: **10-03-2000**

Table of Contents

[X] Check here if appendix is included

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

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.

Principal Investigator: Rubhana Raqib

Project Name: "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". The proposed study is an addendum to the above mentioned study.

Total Budget 68,800 USD Beginning Date As soon as possible (One year) Ending Date

Several field studies have confirmed the important role of zinc in immunity and risk of infection. Controlled trials have demonstrated therapeutic effect of zinc on diarrheal episodes, preventive effects on the incidences of diarrhea and acute lower respiratory tract infections (ALRI), and positive effects on physical growth. Evidence of improvement of cellular immune status with zinc supplementation is also present. These studies suggest that supplementation with zinc may markedly improve the protective immune responses against these infectious diseases thereby reducing the severity and episodes of the diarrhea and ALRI. In humans, iron deficiency is associated with an increased risk of infection and its prevention/ or treatment may be expected to lower the incidence of common infections. Several studies have shown that individuals with iron deficiency show impairment of cell-mediated immunity, humoral immunity and phagocyte microbicidal function. The benefit of micronutrient mixture supplementation is controversial. However, if zinc, iron or micronutrient mixture have positive effect on the immune response, it may be adopted as a public health measure to enhance the efficacy of vaccines. The ongoing study 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") (Protocol #99-014) is a community based, 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 will be studied. (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 (UNICEF mix), and (v) riboflavin only (placebo) will be studied. The outcome variables that will be measured are: (a) iron status, (b) zinc status, (c) copper status, (iv) diarrheal morbidity, (v) growth, and (f) cognitive, psychomotor and behavioral development. Eight hundred infants will be selected from the Matlab villages and will be enrolled in the study over a period of six months. The objective of the present protocol is to study the humoral and cell-mediated immune responses in children before and after micronutrient supplementation. The impact of iron and / or zinc supplementation and micronutrient mixture supplementation on the immune status of infants will be studied in the proposed addendum. Infants from selected Matlab villages meeting the inclusion and exclusion criteria as given in the original protocol will be enrolled in the parent study. In addition to the criteria set for the original protocol, subjects who develop any infection including ARI or diarrheal diseases will be excluded from the proposed study since the infection may have an effect on the immune responses of these subjects. Before and 6 months after supplementation, the following will be studied: (1) The levels of total and specific IgA (response against measles/oral Polio vaccine) in serum and secretory IgA in stool. (2) Thymidine uptake assay of lymphocytes after stimulation with mitogens and measurement of IL-2 levels in the culture supernatant to study proliferation responses. (3) Multitest CMI skin test and (4) Phenotypic changes in the peripheral blood mononuclear cells to study the cell-mediated immune response in infants. (5) Complement response (C3, C5) in infants. Long term supplementation of iron and/or zinc or micronutrient mix in infants six months onward will allow us to understand whether any of these supplementation alone or in combination improves humoral immune responses (ie. whether there is an increase in levels of total IgA and complements and specific IgG response against vaccines (measles/oral polio vaccine) in serum and s-IgA in stool), and cellular immune status (CMI, proliferation response).

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Rubhana Raqib	Assistant Scientist / Immunology	Prinicipal Investigator
2. Abdullah H Baqui	Head, CHP/ Epidemiology	Co- Prinicipal Investigator
3. Md Yunus	Head, MHRP/ Epidemiology	Co-investigator
4. K Zaman	Associate Scientist / Epidemiology	Co-investigator
5. Firdausi Qadri	Senior Scientist / Immunology	Co-investigator
6. George Fuchs	Division Director, CSD/ Nutrition	Co-investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

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

A. Long term supplementation of iron and/or zinc or micornutrient mix in infants six months onward will:

1. increase the levels of total IgA levels in serum (in infants who will receive zinc alone) and s-IgA in stool (in infants who will receive zinc alone or iron alone or in combination)
2. increase specific IgG response against vaccines (measles or oral polio vaccine) (in infants who will receive zinc alone or iron alone or in combination)
3. improve cellular immune status by enhancing the proliferative response against mitogen (PHA) and response against multiple antigens (in infants who will receive zinc alone or iron alone or in combination)
4. enhance the numbers of Pan T cells (CD3), T helper cells (CD4) (in infants who will receive zinc alone) and natural killer cells (CD56 / CD16) (in infants who will receive iron alone) in systemic circulation
5. activate complement components in blood (in infants who will receive zinc alone)

B. Infants who will receive the micornutrient mix will have an overall positive effect on the immune status as described above.

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).**

To study the following before and 6 months after supplementation:

1. The levels of total and specific IgA (response against measles/oral Polio vaccine) in serum and secretory IgA in stool.
2. To study the proliferative responses of lymphocytes to mitogens and to measure IL-2 levels in the culture supernatant.
3. Multitest CMI skin test will be performed to study the cell-mediated immune response in infants.

4. To study phenotypic changes in the peripheral blood mononuclear cells.
5. To study the complement response (C3, C5) in infants.

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).**

Original protocol:

The above study (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") (Protocol #99-014) is a community based, 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 will be studied. (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 (UNICEF mix), and (v) riboflavin only (placebo) will be studied.

The outcome variables that will be measured are: (a) iron status, (b) zinc status, (c) copper status, (iv) diarrheal morbidity, (v) growth, and (f) cognitive, psychomotor and behavioural development. Eight hundred infants will be selected from the Matlab villages and will be enrolled in the study over a period of six months. Selection criteria are described in the original

proposal. 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 5 study groups using a block randomization procedure. Trained community health workers (CHW) will visit the infants every week to feed the infants with the assigned supplements as well as collect morbidity data. Growth, weight, length, and MUAC of the infants will be measured at enrollment and thereafter every two months. Finger prick blood samples will be collected from each study infant at the start and end of the follow-up for hemoglobin test. About 3 ml of venous blood samples will be collected by a trained nurse from 50% of the study infants at the start and the end of the follow-up (6 months and 12 months) and will be tested for serum transferrin receptor (sTfR), zinc and copper. An assessment of mental, psychomotor and behavioural developments will be carried out in a 50% sample of study infants at 6 months and 12 months by a trained psychologist using Bailey" Scale of Infant Development, Version 2.

Addendum.

Micronutrients, the immune system and resistance to infection form an absolute trinity. One influences the other. The severity of the immunological impairment depends on the extent and nature of the nutritional status, the presence of infections and the age of the individual (1). Infection by itself results in micronutrient losses and often produces immunosuppression. In recent years greater attention has been devoted to the possible effects of individual nutrients on risk of infection.

Recent studies showed a significant link between malnutrition and cell-mediated immune deficiency and were found to be independent risk factors for the occurrence of diarrhea and the combination responsible for acute respiratory infections in Bangladesh (2-4). There was a suggestion that diarrhea could lead to zinc deficiency, which in turn could impair immune responses and hence increased risks of infection. Good and Lorenz (5) showed that it was difficult to reproduce any severe immune deficiency by inducing protein energy malnutrition in animals. Only by depriving them also of zinc could they impair cell-mediated immune reactivity. It was considered that protein energy malnutrition might often include various micronutrient deficiencies as well that explain the many abnormal immune functions. It is now well established that zinc deficiency is widely prevalent in the Asian subcontinent (6-8). Several studies using controlled trials have demonstrated therapeutic effect of zinc on diarrheal incidences, preventive effects on diarrhea and ALRI morbidities, and positive effects on physical growth (7-14). Evidence of improvement of cellular immune status with zinc supplementation is also present (15-17). These studies suggest that supplementation with zinc may markedly improve the protective immune responses against these infectious diseases thereby reducing the severity and episodes of the diarrhea and ALRI. Zinc plays an important role in immunity since there are hundreds of zinc-dependent enzymes, many of these are critical for cellular metabolic pathways. Thus, zinc deficiency results in profound immunodeficiency. Zinc deficiency impairs cell regeneration, instability of cell membrane, epithelial immunocompetence with reduced cell-mediated immunity (reduced lymphocyte proliferation response, decreased production of TH1 cytokines, decreased T helper cell/ T cytotoxic cell ratio, impaired or reduced macrophage and natural killer cell function and decreased antibody production after challenge with T dependent antigens, decreased thymulin production) (18-21). However the role of zinc in antibody production is not clear. Studies in animals have shown that zinc supplementation increased serum IgG and IgM responses (22). Serum IgA levels significantly increased in malnourished children supplementated with zinc (17). Zinc is also known to activate complement C3 and C5 *in vitro* and in malnourished animal models and heightens resistance against bacterial infections and increased complement response (23, 24). Since zinc is important for stability of cell membrane, if the integrity of intestinal epithelium is disrupted due to zinc deficiency, it is likely to influence loss of s-IgA from the gut.

Several studies have shown that individuals with iron deficiency show impairment of cell-mediated immunity (delayed cutaneous hypersensititivity responses, T lymphocyte proliferation response to mitogens) and phagocyte microbicidal function. Recently, iron deficiency has been shown to reduce natural killer activity, reduce production of cytokines such as IL-2 and IFN-γ (25). In iron-deficient rats, sIgA and IgM containing cells in the intestinal mucosa was significantly lower than in iron-sufficient rats accompanied by reduced mitotic index of crypt epithelial cells and the abnormality was reversed after a week of iron supplementation (26). Another study reported that iron fortified milk significantly increased the production of sIgA in the gut, thereby enhancing the development of immunologic competence in early infancy (27). Deficiencies of iron, zinc and copper have been shown to impair cellular immune responses as evident by lower production of interleukin 2 (IL-2) by T lymphocytes *in vitro* and supplementation with the respective micronutrients was able to increase generation of IL-2 (25, 28, 29).

The benefit of micronutrient mixture supplementation is controversial. If zinc, iron or micronutrient mixture have positive effect on the immune response, it may be adopted as a public health measure to enhance the efficacy of vaccines. In fact, an ongoing study at ICDDR,B (Effects of vitamin A and zinc supplementation on the immune response to oral cholera vaccination in Bangladeshi children, PI: Dr John Albert, protocol: 9-8-001) aims to test the

hypothesis that supplementation with zinc, vitamin A or both can improve vibriocidal as well as antigen specific antibody response to killed 01 Cholera vaccine. Development of the immune system can be assessed by testing the antibody response to immunizations with measles vaccine that is given at the age of 9 months. Till date, there is no report on the effect of zinc supplementation on antibody response to measles vaccination. There are studies that indicate that simultaneous administration of measles vaccine and vitamin A supplements had either no negative effect on measles immunity or a significant positive effect (30, 31). On the other hand, reports of simultaneous vitamin A supplementation interfering seroconversion to live measles vaccine in infants is also present (32). There is evidence that infant formula fortified with nucelotides can significantly enhance concentration of antibody against *Haemophilus influenzae* type b and diptheria during infancy (33) suggesting that dietary factors play a significant role in the antibody responses of infants to immunization.

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 Subjects

Infants from selected Matlab villages meeting the inclusion and exclusion criteria as given in the original protocol will be enrolled in the parent study. In addition to the criteria set for the original protocol, subjects who develop any infection including ARI or diarrheal diseases will be excluded from the proposed study since the infection may have an effect on the immune responses of these subjects.

Outcome Variables and Sample size Determinants

The following outcome variables will be measured:

(i)	Humoral responses:	IgA in serum
		Serum IgG responses to measles/Polio vaccines
		sIgA in stool
		Complement components C3 and C5 in serum
(ii)	Cell mediated immunity:	Proliferative response of peripheral lymphocytes to mitogens
		Interleukin 2 in culture supernatant of proliferative lymphocytes
		Phenotypic changes in the peripheral blood

Table 1. Sample size calculations:

Outcome variables	Expected levels in the control group	Expected level in the treatment group (expected % improvement/ reduction)	*Sample size required in each group	References
1. IgA in serum, mg/L	81±32	111±26	19	17
2. s-IgA in stool, g/L (for zinc)	0.05±0.01	1.1±0.45	3	36
3. s-IgA in stool, mg/dl (for iron)	0.05±0.01	7.4±0.25	2	27
4. Antibody titers against Polio virus vaccine	169	346	99	33
5. §Response to PHA	31±9	58±13	4	17
6. IL-2 in culture supernatant, units/ml	106.5±99	137.3±67	149	28
7. CMI, % of anergic children	67%	47%	131	15
8. Phenotypic changes a) CD3	0.43±0.53	2.09±1.68	12	28
b) CD4	0.30±0.39	1.43±1.24	14	28
c) CD8	0.13±0.17	0.76±0.54	9	28

§Stimulation index, mean±SD. A loss of follow-up of 20% has been included. *Sample size required in each group of infants supplemented with either zinc alone or iron alone, or zinc and iron in combination or the multimix. Allowance for 10% drop-out has been made. Since sample size is different for each variable, for convenience we decided that for variables 1, 2, 3, 5, and 8, sample size is 20. Sample size for variables 4, 6 and 7 will remain the same as shown in the table.

Methodology

In the original project, blood (3 ml) samples will be collected before micronutrient supplementation and 6 months later. In the present proposal, additional 1ml of blood will be collected at the same time points, therefore the total blood volume will be 4 ml. Mononuclear cells will be separated from total blood and used for various purposes. Stool samples will be collected at the time of blood collections.

Blood:

Lymphocytes

Blood (4 ml) will be collected in heparinized tubes. Lymphocytes separated from venous blood upon Ficoll-Hypaque separation will be used for proliferation response to mitogens (PHA) and for phenotyping of lymphocytes by flow cytometry. Lymphocytes will be frozen and carried in

liquid nitrogen to the Karolinska Institutet at the Division of Infectious Diseases, Huddinge University Hospital where phenotying will be performed.

Plasma

Total IgA contents will be determined in plasma using ELISA with pooled human Swedish milk with a known IgA concentration of 1 mg/ml as a standard (34). In brief, Nunc plates will be coated with affinity pure goat antihuman IgG as a concentration of 1 μg/ml in PBS(phosphate buffered saline) (100 μl/well).and incubate the overnight at room temperature. Plates will be washed 2 times with PBS and blocked with 1% BSA-PBS (bovine serum albumin-phosphate buffered saline) (200 μl/ml) and will be incubated for 30 min at37^{0}C. After incubation, plates will be washed 3 times with PBS-Tween and once with PBS. Standard Swedish milk (2 μg/ml) and samples (100 μl/well) will be added to the specific wells. In case of samples initial dilution will be 1: 500 and then 3 fold serial dilution continued. . Plates will be incubated for 90 minutes at room temperature. After washing as above, conjugate Goat anti human-IgA-HRP (horse radish peroxidase) (1:4000 in 0.1% BSA-PBS-Tween, 100 μl/well) will be added and incubated for 1 hrs in room temperature. After washing 3 times, OPD substrate will be added and plates will be read after 20 minutes of development at 450nm.

Antibody titers to measles immunization will be carried out using ELISA (31). Complement C3 and C5 in plasma will be measured using commercially available kits (Amersham Pharmacia Biotech, England). IL-2 will be measured in the culture supernatant from mitogen-stimulated cells after proliferation using commercially available kits (R&D Systems, Minneapolis, USA).

Stool

Secretory IgA response will be determined in stool using the standard ELISA (35). In brief, Nunc plates will be coated with secretory component (1:2000 in carbonate buffer) (100 μl/ml) and incubate the overnight at room temperature. Plates will be washed 3 times with PBS-Tween and blocked with 1% BSA-PBS (bovine serum albumin-phosphate buffered saline) and will be incubated for 60 min at room temperature.. After incubation, plates will be washed 3 times with PBS-Tween. Standard Swedish milk (2 μg/ml) and samples (100 μl/well) will be added. Initial dilution will be 1:50 and then 3 fold serial dilution will be done. Plates will be incubated O/N at room temperature. After washing as above, conjugate s-IgA-HRP (horse radish peroxidase) (1:1000 in 0.1% BSA-PBS-Tween, 100 μl/well) will be added and incubated for 2 hrs in room temperature. After washing 3 times, OPD substrate will be added and nm after 20 minutes of development, 1.0 M sulfuric acid (25 μg/well) will be added to the plates to stop reaction and plates will be read at 492.

Skin test

Multitest CMI skin test will be performed using the commercial multiple antigen skin test kit (Multitest CMI, Institute Merieux, Lyon, France).

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

Immunological assays including enzyme linked immunosorbant assays (ELISA), lymphocyte proliferation assay, will be carried out at ICDDR,B since the techniques have been standardized and the equipment needed for carrying out the assays are available in ICDDR,B. However, to perform sterile work, a biohazard hood will be required at Matlab. For separating lymphocytes from blood, a refrigerated table-top-centrifuge will also be needed at Matlab. An ultra low freezer (-80° C) will be required to store lymphocytes after separation from whole blood. These lymphocytes will be phenotyped later by flow cytometry. Since flourescent associated cell sorter (FACS) is not available at ICDDR,B, frozen cells have to be carried in dry ice (-70° C) to Sweden. FACS will be available to the PI to perform flow cytometric assay (phenotyping) at the Division of Infectious Diseases, Karolinska Institutet, Sweden.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

Baseline characteristics of the different supplementation/treatment and comparison groups will be examined for group comparability as described in the original protocol.

To examine the effect of individual micronutrient i.e. zinc or iron on the immune parameters between the pre and post supplementation days, Wilcoxon-signed Rank test will be used. To examine the proportion of subjects responding to a particular supplement, Chi-square test will be performed. To examine the potential additive and multiplicative effects of providing zinc, iron and other micronutrients on the immune parameters, multiple regression analyses will be performed.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Permission to study infants between 6-11 months of infants for the parent protocol has been obtained and the study has already started. Permission to draw 3 ml of blood from infants has been approved. From this 3 ml of blood, mononuclear cells will be obtained to do proliferation assay, and phenotyping of lymphocytes. Determination of total IgA and specific IgG and complements, approximately 500 µl of serum will be required. For this purpose an additional 1 ml of blood will be required.

The ethical implications of this study are outlined below:

1. There will be a minor bruise while drawing of blood and a probability though very little of risk of infection related to blood collection.

2. All necessary precautions will be taken and sterile techniques will be used to collect blood samples to reduce risk of infection.

3. Infants, 6-11 months of age usually have a blood volume of approximately 350 to 400 ml. Additional 1 ml of blood plus 3 ml in the original protocol therefore, will not be harmful to the infant.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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2. Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M and Siddique AK. 1993. Malnutrition, cell-mediated immune deficiency, and diarrhea: a community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 137:355-365.
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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training program.

Research findings will be published in international journals to make the results available to all researchers in the relevant fields and will be presented in international conferences. In developing countries like Bangladesh infants often suffer from multiple infections and dietary insufficiencies of multiple micronutrients is also highly prevalent. Infection by itself results in micronutrient losses and often produces immunosuppression. Results obtained from this study may help in better understanding the effects of supplementation of zinc, iron or micronutrient mixture on the immune system. If supplementation with zinc, iron or micronutrient mixture have positive effect on the immune response, it may be adopted as a public health measure to improve the nutritional status of these infants. Also it has been speculated that micronutrient deficiencies may suppress the development of specific immune response to vaccination or may lead to an anergic state. Thus, if zinc, iron or micronutrient mixture supplementation have positive effect

on the development of protective immune responses, it may be adopted as a public health measure to enhance the efficacy of vaccines.

Biography of the Investigator

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name:	Rubhana Raqib	**Position:**	Assistant Scientist, Immunology,
Date of Birth:	19 October, 1961		LSD, ICDDR,B, Dhaka, Bangladesh

Academic Qualifications

Institution and Location	Degree	Year	Field of Study
Karolinska Institutet, Sweden	PhD	1995	Immunology
Dhaka University	M.Sc.	1988	Biochemistry
Dhaka University	B.Sc.	1985	Biochemistry

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1. For masters degree, research acitivities involved extraction, purification and study of the immunogenic properties of outermembrane proteins from *Shigella dysenteriae* type 1 and *Shigella flexneri* strains using immunoelectrophoresis, SDS-PAGE and Western blot.

2. For phD. dissertation, research activities were focussed on the study of the pathogenic mechanisms and immune responses in adult patients with shigellosis. Samples such as plasma, peripharal blood mononuclear cells, stools and rectal biopsies were collected from patients and healthy subjects and were analysed for cytokines (protein and mRNA), cytokine receptors and phenotypes of various cells and activation markers. The techniques used were ELISPOT, ELISA, immunohistochemistry, quantitative analysis of video microscopic images and *in situ* hybridization.

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Detailed Budget

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.

Name of PI: Rubhana Raqib; Name of Co-PI: Abdullah H Baqui
Name of Divisions: (1) LSD (2) PHSD

Funding Source: Being sought Direct Cost: 68,800 US$

Starting Date: As soon as possible Closing Date: One year from starting

Sl. No	Account Description		Salary Support		
	Personnel	**Position**	**Effort %**	**Salary**	**One Year**
1	Rubhana Raqib	NOB	25	970	3,000
4	Research Officer, at ICDDR,B	GS-5	100	349	4,200
5	Research Officer, at Matlab	GS-5	40	349	1,700
6	Lab attendent	GS-1	100	170	2,400
	Sub Total				**11,300**
	Travel				
8	International Travel (trip to KI)				5,000
	Sub Total				**5,000**
	Laboratory Analysis				
9	Immunological Assays				
	Ficoll-hypaque blood separation, buffers				1,000
	Complement and IL-2 cytokine ELISA kits				5,500
	Mitogen, antigens, monoclonal antibodies for flow cytometry				4,000
	Reagents for thymidine uptake assay, cell culture reagents etc				5,000
	Antibodies, and reagents for ELISA				4,500
10	Laboratory Supplies				
	Plasticware, glassware, Chemicals and media, ELISA plates, stool extraction tubes, sterile microfilters etc				5,000
	Sub Total				**25,000**

	Other Contractual Services	**One Year**
14	Printing and Publication	1,500
	Communication, repair, maintenance	800
15	Dry ice and freight charges	1,000
	Sub Total	**3,300**
	Interdepartmental Services	
16	Pathological Tests	600
17	Microbiological tests	600
18	Biochemistry Tests	500
	Sub Total	**1,700**
24	**Capital Expenditure** Table-top centrifuge, refrigerated Biohazard hood Ultra low Freezer, –83° C	 7,500 10,000 5,000
	Sub Total	**22,500**
	TOTAL DIRECT COST	**68,800**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Two research officers are needed, one for the Matlab lab (40%) and one for the Immunology lab (100%) in ICDDR,B to carry out immunological assays. A lab attendant will be required at the Dhaka lab.

Capital equipment cost includes a table top refrigerated centrifuge, a biohazard hood and an ultra low freezer (–83° C). The table top refrigerated centrifuge will be needed for separating lymphocytes from blood. The biohazard hood will be required to perform sterile work including cell proliferation assay. The ultra low freezer will be required to store lymphocytes. These lymphocytes will be phenotyped later by flow cytometry. These pieces of equipment are essential for carrying out immunological assays in Matlab laboratory. Once this laboratory is equipped, it will be possible to carry out future immunological studies including vaccine immunogenicity studies in Matlab.

International travel is required for carrying out flow cytometric assay at the Division of Infectious Diseases, Karolinska Institutet, Sweden.

Annex - 1

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 4 ml blood will be collected 2 times from 50% of the study infants.

Annex - 2

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 micronutient 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 micronutient 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 micronutient 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. CMI (cell mediated immune) skin test will also be performed. 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 4 ml venous blood samples and stool samples will be collected from the study infants at the start and end of the follow-up. Blood will be tested for serum transferrin receptor (sTfR), ferritin, zinc, and copper and the secretory IgA(S-IgA) will measured in stool. 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 collaction 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 /LTI of parent: _____ Date: _____

Name of witness:_____ Signature of witness:_____

গবেষণার নাম: A community-based, randomised, controlled trial to assess the efficacy of iron and/or zinc or a micronutrient mix supplement to reduce anaemia and morbidity and to improve growth and development in Bangladeshi infants.

Investigators: Abdullah H. Baqui and others, ICDDR,B.

সম্মতি-পত্র

গবেষণায় দেখা গেছে যে, উন্নয়নশীল দেশে শিশুদের খাদ্যে আয়রন ও জিঙ্ক-সহ অন্যান্য মাইক্রোনিউট্রিয়েন্ট-এর ঘাটতি থাকে। ফলে অনেক শিশুর ৬ মাস বয়সের মধ্যেই মাইক্রোনিউট্রিয়েন্ট, বিশেষ করে আয়রণ ও জিঙ্ক-এর ঘাটতি দেখা দেয়। অধিকতর অল্প বয়সে আয়রণের ঘাটতি হলে শিশুদের রক্তশূণ্যতা দেখা দেয়, শারীরিক ও মানসিক বৃদ্ধি ব্যহত হয়। গবেষণায় দেখা গেছে যে, ডায়রিয়া চলাকালীন সময়ে এবং ডায়রিয়া ও নিউমোনিয়া প্রতিরোধে জিঙ্ক-এর উপকারিতা আছে। এছাড়াও, জিঙ্ক শিশুদের শারীরিক ও মানসিক বৃদ্ধি এবং রোগ প্রতিরোধ ক্ষমতায় উপকারী ভূমিকা রাখে। কাজেই, যে সমস্ত দেশে খাদ্যের মাধ্যমে মাইক্রোনিউট্রিয়েন্ট-এর ঘাটতি পুরণ করার জন্য কোনো তাৎক্ষণিক উপায় নেই, সেখানে শিশুদের রক্তশূণ্যতা ও রোগ-প্রতিরোধে এবং শারীরিক ও মানসিক বৃদ্ধিতে মাইক্রোনিউট্রিয়েন্ট সাপ্লিমেন্ট-এর প্রয়োজনীয়তা ও সম্ভাব্যতা নিরূপণ করা দরকার।

এই গবেষণাতে শিশুদের বিভিন্ন ধরণের মাইক্রোনিউট্রিয়েন্ট ফরমুলার উপকারিতা পরীক্ষা করে দেখা হবে। আপনি একজন শিশুর পিতা/মাতা/অভিভাবক হিসেবে আমরা আপনাকে অনুরোধ করছি যে, আপনি আপনার শিশুকে এই গবেষণা কাজে অংশগ্রহণ করতে অনুমতি দিবেন। এই গবেষণা আই-সি-ডি-ডি-আর, বি-র সহায়তায় পরিচালিত হবে। এটা আপনার বোঝা অত্যন্ত প্রয়োজন যে, (ক) এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক, এবং (খ) আপনি যে কোনো সময়ে আপনার শিশুকে এই গবেষণা হতে সরিয়ে নিতে পারেন। যে কোনো ক্ষেত্রেই, আই-সি-ডি-ডি-আর, বি-তে (কলেরা হাসপাতালে) আপনার শিশুর বা আপনার পরিবারের কারো কোনো সময়ে প্রয়োজনীয় চিকিৎসার কোনো ত্রুটি হবে না।

এই গবেষণায় অংশগ্রহণ করলে আপনার শিশু ৬ মাস বয়স থেকে আরও ৬ মাস প্রতি সপ্তাহে একবার করে নিম্নলিখিত মাইক্রোনিউট্রিয়েন্ট ফরমুলার যে কোনো একটি পাবে: (১) ২০ মি:গ্রা: আয়রণ ও ১ মি:গ্রা: রিবোফ্লেবিন, (২) ২০ মি:গ্রা: জিঙ্ক ও ১ মি:গ্রা: রিবোফ্লেবিন, (৩) আয়রন, জিঙ্ক ও রিবোফ্লেবিন, (৪) মাইক্রোনিউট্রিয়েন্ট মিক্সচার, বা (৫) শুধুমাত্র রিবোফ্লেবিন। আপনার শিশু কোনটি পাবে তা লটারী করে ঠিক করা হবে, এখানে আমাদের কোনো হাত নেই।

একজন প্রশিক্ষণপ্রাপ্ত মাঠকর্মী সপ্তাহে একদিন আপনার শিশুকে দেখতে যাবেন, শিশুর গত এক সপ্তাহের অসুস্থতার বিবরণ সংগ্রহ করবেন, এবং শিশুকে উপরোক্ত যে কোনো একটি (লটারীতে তার নামে যেটি উঠেছে) সিরাপ খাওয়াবেন। শিশুর বৃদ্ধি পরিমাপের জন্য গবেষণার শুরুতে এবং তার পরে প্রতি দুই মাস পর পর শিশুর ওজন, দৈর্ঘ্য এবং মধ্য-বাহুর মাপ নেওয়া হবে। শিশুর ত্বকে সি এম আই রোগ প্রতিরোধ ক্ষমতা দেখা হবে। গবেষণার শুরুতে এবং শেষে রক্তে হিমোগ্লোবিন মাপার জন্য শিশুর আঙ্গুল থেকে এক ফোঁটা রক্ত নেয়া হবে। এছাড়া, গবেষণার শুরুতে এবং শেষে শিশুর শরীরের শিরা থেকে চা চামচের এক চামচ-এরও কম (৪ মি: লি:) রক্ত এবং মল নেয়া হবে। শিশুর রক্তে মাইক্রোনিউট্রিয়েন্ট, বিশেষ করে আয়রণ ও জিঙ্ক পরিমাপ করা হবে এবং মলে অন্ত্র নিঃসৃত এন্টিবডি (আই-জি-এ) পরিমাপ করা হবে। একজন প্রশিক্ষণপ্রাপ্ত মনস্তত্ত্ববিদ শিশুর ৬ মাস ও ১২ মাস বয়সে শিশুর শারীরিক ও মানসিক বৃদ্ধি পরিমাপ করবেন। আপনার শিশু যদি উক্ত অর্ধেকের মধ্যে না পড়ে তবে তার রক্ত পরীক্ষা বা শারীরিক ও মানসিক প্রবৃদ্ধি পরীক্ষার প্রয়োজন হবে না।

এই গবেষণায় অংশগ্রহণ করলে আপনার পরিচয় প্রকাশ হয়ে পড়ার কোনো সম্ভাবনা নেই। আপনার বা আপনার শিশুর নাম বা পরিচিতিমূলক কোনো তথ্য বাইরে কোথাও প্রকাশ করা হবে না। শুধুমাত্র কাজের প্রয়োজনে আমাদের নিজস্ব প্রতিনিধি এই সব কাগজপত্র পরীক্ষা করতে পারেন।

আপনি যদি আপনার শিশুকে এই গবেষণায় অংশগ্রহণ করতে দিতে রাজী থাকেন তবে অনুগ্রহপূর্বক এই সম্মতিপত্রে স্বাক্ষর করুন বা টিপসহি দিন।

সম্মতি

উপরোক্ত গবেষণা আমাকে পুরোপুরি ব্যাখ্যা করা হয়েছে। আমার সকল প্রশ্নের সন্তোষজনক উত্তর দেয়া হয়েছে। আমি আমার শিশুকে এই গবেষণায় অংশ নিতে অনুমতি দিতে রাজী আছি।

আমি (নাম): _____ আর-আই-ডি: _____ স্বেচ্ছায়

আমার শিশু (নাম) _____ সি-আই-ডি: _____-কে তার অভিভাবক হিসেবে এই গবেষণায় অংশগ্রহণ করতে দিতে সম্মতি দিলাম।

পিতা/মাতা/অভিভাবক-এর স্বাক্ষর/টিপসহি: _____ তারিখ: _____

স্বাক্ষীর নাম: _____ স্বাক্ষীর স্বাক্ষর: _____