Attachment I.
(FACE SHEET)

~~Library~~ ~~Revised~~

Date 29/8/96

ETHICAL REVIEW COMMITTEE, ICDDR,B.

EC

Principal Investigator Dr. S.K. Roy

Trainee Investigator (if any)

Application No. 95-030

Supporting Agency (If Non-ICDDR,B)

Title of Study Impact of zinc and calcium supplementation in children with shigellosis

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of 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) Minors or persons under guardianship — Yes No
2. Does the study involve:
   - (a) Physical risks to the subjects — Yes No
   - (b) Social Risks — 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, birth 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 study — Yes No
   - (b) Procedures to be followed including alternatives used — Yes No
   - (c) Physical risks — 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 parent or guardian (if subjects are minors) — 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 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. Examples 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 Cttee. for review.

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

SKRoy
Principal Investigator

Trainee

CHECK-LIST FOR SUBMISSION OF PROPOSALS TO THE RESEARCH REVIEW COMMITTEE (RRC)

[Please tick (√) the appropriate box]

1: Has the proposal been reviewed, discussed and cleared at the Division level ?

Yes ☑

No ☐

If the answer is 'NO', please clarify the reasons: ______

2. Has the proposal been reviewed externally ?

Yes ☑

No ☐

If the answer is 'NO', please explain the reasons: ______

3. Does the proposal address gender issues ?

Yes ☐

No ☑

If the answer is 'NO', please give the reasons:

Both sexes are equally involved

4. Has a funding source been identified ?

Yes ☑

No ☐

If the answer is 'YES', please indicate the name of the donor:

NUTRICIA FOUNDATION, NETHERLANDS

Contd...../.

5. Whether the proposal is a collaborative one ?

Yes ☑

No ☐

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

Dr. M.A. Khaled, University of Alabama, USA

6. Has the budget been cleared by Finance Division ?

Yes ☑

No ☐

If the answer is 'NO', reasons thereof be indicated:

7. Does the study involve any procedure employing hazardous materials, or equipments?

Yes ☐

No ☑

If the answer is 'YES', fill the necessary form.

Date 29/8/96

Signature of the
Principal Investigator

1. **Title**: Impact of zinc and calcium supplementation in children with shigellosis.

2. **Principal investigator**: Dr. S. K. Roy

3. Co- principal investigators: Dr. G.Fuchs
Dr. Ali Miraj Khan
Dr. A. Khaled
Dr. T. Azim

Collaborative investigator: Dr. Josiane Arnold
Laboratories de Biochimie C
Greenoble, France

4. Starting date: As soon as funds are available

5. Date of completion: 2 years from the beginning

6. Total direct cost: US $ 129,353

7. Source of fund

8. Approval by the Division Director, CSD ______________

Approval by the Director, ICDDR,B ______________

Approval By the Research Review Committee ______________

Approval By the Ethical Review Committee ______________

29 th August, 1996

9. Abstract:

The aim of this study is to evaluate the role of selected trace elements (Zinc, calcium & both together) on clinical course (reduction in protein breakdown, severity of infection, linear growth) central immune mediators (CMI response, IL-1, IL-6) and pathophysiologic sequelae (oxidative stress and nitric oxide production) in shigellosis in children. In this study, children aged between 1 and 3 years of either sex with symptomatic shigellosis (loose stool with mucus and blood) will be included. Informed consents will be obtained from the parents of the participating patients before entry into the study. They will be admitted to the hospital for 5 days and treated with pivmecillinum 60 mg/kg of body weight/per day in four divided doses along with one of four syrups according to a random numbers table (syrup containing MV+ZINC or MV+Calcium or MV+Zinc+Calcium or MV alone). The syrups will be given for a total period of two weeks. Patients will be rehydrated using I.V./oral fluid as needed. The treatment effects will be assessed by changes in clinical symptoms, stool characteristics, growth enhancement, immune status, nitric oxide production, free radical generation, and reduction of muscle protein break down by measuring urinary 3-methyl histidine excretion. If the supplementation of zinc and calcium are found to be beneficial, it will provide a simple and useful supplement during antimicrobial treatment for shigellosis in children to reduce severity and duration of shigellosis and subsequent growth faltering.

Objectives of the study:

To evaluate the effect of selected nutrients in the treatment of shigellosis in children.

Specific objectives:

To examine the specific roles of selected trace elements

Zinc and/or Calcium on

a) Clinical course, b) protein breakdown,
c) immune indices, d) growth faltering and
e) host oxidative stress in shigellosis in children.

Background information:

[Acute diarrhoea remains a major cause of childhood mortality and morbidity in the developing countries. The delayed, and possibly more deleterious, effect of diarrhoea is malnutrition. Diarrhoea and malnutrition are frequently seen as combined problem in children of the developing countries.] Although Briend et al

(1990) showed no impact of acute watery diarrhoea on weight gain, they cautioned too rapid a dismissal of the possibility of an impact on linear growth. They noticed significant growth reduction at 3 months when diarrhoea occurred at the beginning of the observation period, and even at the 6 month interval, 30 % of children remained stunted. Henry et al. further confirmed that dysentery causes stunting in children (Henry 1987). Black showed that children infected with E. coli (ST) had been stunted (Black et al. 1984). One of our previous studies showed that (Behrens et al 1990) supplementation of zinc during diarrhoea enabled a 25% increase in linear growth predominantly over the fourth to nine week after presentation of acute diarrhoea. Accordingly, It was proposed a zinc deprivation cycle occurs during diarrhoea, which may be associated with post diarrhoeal growth faltering in children.

[Micronutrient deficiencies are associated with increased diarrhoeal prevalence in children. Essential micronutrients include vitamin A, zinc ,folate, iron,iodine and copper.] However, the role of certain of these micronutrients on physical growth of children and their morbidity and mortality has not been adequately evaluated.

Role of Zinc

Immunity

Diminished in vitro responses to T cells and combined T and B cells to mitogens (PHA and CON-A) have been demonstrated in lymphocytes from zinc- deficient rats,but pair-fed controls showed normal responses (Gross et al,1979). [It has been reported that malnourished and zinc deficient children had earlier clinical recovery from acute and persistent diarrhoea with zinc supplementation (Roy et al 1992, Sachdev et al 1993).]

There is a recognition of the critical role of dietary intake and nutritional status in modulating immune responses and susceptibility to infection (Scrimshaw et al. 1968 and Chandra et al 1977).Deficiency of calcium leads to compromised immunological status of the host because calcium has definite regulatory role of lymphocyte function and activation of complement (Beisel WR,1982). Calcium has shown to increase linear growth after supplementation in Srilanka (LSHTM, 1993).

[In malnourished children, there is a significant depression of cell-mediated immunity. CHI depression have been found in individuals with predominant deficiencies of iron (Chandra RK,1975,1977 & 1980),folate (Gross et al,1975) and zinc (Chandra RK,1980). In an animal model it has been observed that low zinc concentration decreases the number of helper T-cell and increase the suppressor activity of immune response (Gross et al 1979).] A recent study was conducted by one of the co-investigator of this study which has shown that TNF-α rises high in shigellosis and the fall is dramatic by 3 days median 1348 to median 1 which would be inconvenient marker for micronutrient response in shigellosis (Azim T 1995). However it is difficult to evaluate the effect of deficiency of micronutrient on clinical course and immune response in severe disease like shigellosis.

Malnourished children usually having micronutrient deficiency are more prone to oxidative damage induced by the reactive oxygen species thus producing a state of oxidative stress. Oxidative stress induced by reactive oxygen species (ROS) has been implicated in many human diseases in recent years (Sies H, 1991). These ROS are usually scavenged by certain endogenous enzymes such as super oxide dismutase (SOD), catalase, glutathione reductase and peroxide. Moreover, Halliwell (1988) has demonstrated the importance of albumin as an extracellular antioxidant. Hypoalbuminemia is a common feature in malnutrition, a condition which is highly prevalent in children in the developing countries. Huang CJ et al demonstrated in an animal study that protein insufficiency has also been implicated to aggravate lipid peroxidation (Huang 1992).

Shigellosis has been recognized as an important bacterial cause of severe dysenteric illness in children and is often associated with serious complications including hemolytic uraemic syndrome, leukemoid reaction, encephalopathy, protein losing enteropathy malnutrition, and sometimes death (Bennish ML,1991). It has been estimated that nearly 650,000 children die each year because of shigellosis. A substantial number of children who survive however, suffer from severe malnutrition that may be attributed to a combination of loss of appetite, withholding of food, and loss of nutrients in stool (Scrimshaw NS,1977).

Shigellosis causes inflammation in the large bowel. It has been recently discovered that tissue inflammation and cell injury is associated with production of nitric oxide which is a diffusible messenger. NO may act to increase or decrease cellular injury depending on the tissue involved and the physiological circumstance. No production is primarily controlled by inducible versus constitutive expression of three different NO synthases . In humans NO has been shown to down-modulate T-cell proliferation. However recently human macrophages were shown to express iNOS and generate NO in quantities sufficient to kill Lismania parasites.
It is not known how much iNOS activity is increased in shigellosis. And it is worth to explore the potential of micronutrients such as zinc, copper and magnesium on NO regulated cell inflammation. nitrate and Nitrite in blood and urine can be measured to assess as proxy indicator of NOS activity. Recently Islam et al (1995) has shown that iNOS was abundant in shigella biopsy tissue but absent in controls.

The relationship between growth retardation and diarrhoeal diseases has been shown in several studies (Rowland MGM et al,1977 & Tomkins A,1981). In children of developing countries, shigellosis has been shown to have a significant negative effect on linear growth (Black RE et al, 1984 and Henry FJ et al 1987).

Antimicrobial drugs are the mainstay of treatment which have been shown to reduce clinical symptoms and bacterial excretion if the infecting Shigellae are sensitive to the drug. However, antimicrobial treatment alone has several shortcomings. It does not quickly heal mucosal inflammatory lesion, does not prevent development of complications, and finally has problems including cost, patient's compliance, and bacterial resistance. In spite of using effective antimicrobial, there is persistence of clinical symptom including fever, bloody diarrhoea, abdominal cramps, anorexia and discomfort. In addition, nutrient losses can be severe. In view of this, we believe that non-antibiotic adjunctive therapy might hasten the healing of ulceration of the gut as well as give symptomatic relief. Further, adjunctive therapy might be very useful in dysentery due to shigellosis because in shigellosis bacterial overgrowth, mucosal injury or malabsorption of nutrients may lead to the deficiency of both macro and micronutrients. The importance of micronutrients have recently been recognized in the treatment of many intestinal diseases. However, very little is known about the therapeutic role of micronutrients in infective diarrheas including shigellosis.

Hypothesis:

1. Administration of selective micronutrients will improve the clinical course & symptoms of shigellosis in children and reduce stunting after the episode..
2. Administration of micronutrients will improve the immunological status in children suffering from shigellosis.
3. Administration of micronutrients will reduce the lipid peroxidation.

Procedures and Methods:

Study design: Randomized, Clinical trial.

Inclusion criteria:

* Aged between 1 and 3 years.
* Pre-admission duration of illness less than 48 hrs.
* Presence of blood & mucous in stool.
* Patients who have not received drugs known to have effect on shigellosis.
* Nutritional status: between 61-75% of median NCHS standard of wt/age

Exclusion criteria:

* Patients receiving antimicrobial known to be effective against shigellosis.
* Patients with other obvious systemic illness requiring use of other antimicrobial.

antimicrobial.

* Non-availability of consent from the patients.
* Severe Protein energy malnutrition
* Residence difficult for follow up (more than 1 hr by transport)

Study Groups:

Patients will receive multivitamin as they are malnourished and with further risk of malnutrition after shigellosis.

Group-A:	Pivmicellinum + Syrup (Multivitamin + Zn)
Group-B:	Pivmicellinum + syrup (multivitamin + Calcium)
Group-C:	Pivmicellinum + syrup (multivitamin ~~+ placebo~~)
Group-D:	pivmicellinum + ~~Syrup~~ (MV)

Children fulfilling inclusion and exclusion criteria will be randomized into four treatment groups according to a random number table and permutted blocks length 16 will be used. Sealed envelop will be used for treatment of allocations. All patients will receive piivmecillinum 60 mg per kg of body weight in four divided dose along with one of the four syrup according to a random number of table for 5 days. Children of all groups will be followed up in the hospital daily for 5 days and daily records will be kept for the following - stool frequency, temperature, anorexia, abdominal pain, tenesmus, presence of blood in stool, presence of mucus in stool, body weight and height. On admission and daily either a faecal sample or rectal swabs obtained from all children under study will be screened for shigella. At the time of discharge, syrup will be supplied to all the children to take at home for a total of 14 days and also will be advised to report after every 15 days up to three months. On admission, at two weeks and at three months blood will be drawn to perform biochemical estimations.

Ethical consent: Informed written consent will be obtained from the parents of the children after explaining the details of the study procedure.

Sample size: a) Based on linear growth :

Based on a previous study by Behrens et al.(1990), in which the mean ± SD of linear growth in zinc supplementation group of acute diarrhoea patients was 11.8 ± 5.5, it is assumed that by using Pivmicellinum along with micronutrients, a target difference of 30% may be achieved. Hence, the number of patients to be studied in each group is

$$\frac{2 \times (5.5)^2}{(3.5)^2} \times 7.9 = 38$$

The calculated sample size (5% significance, 80% power) is 38 in each group; dropout is 5 in each group. Therefore, a total of 172 patients required to be recruited.

Sample size calculated with other key outcome variables

b) Clinical recovery in reduction days from standard treatment (25% reduction),n=120

c) Change in immunity (20% reduction), n=150

d) Change in 3-methyl histidine excretion (protein breakdown) (25% reduction), n=120

All of the later estimates are smaller than the previous sample size, hence the earlier number would be adequate.

Organization of the trial.

Patients will be selected from the outpatient department of the ICDDR,B and will be admitted into the study ward if they fulfil the admission criteria. The principal investigators with the assistance of a research fellow will take care of the patients for clinical management. The research data will be recorded in coded data forms and will be preserved separately until completion of the study. Patient will be admitted into the study during the morning hours (8:30 am to 12 noon) to enable a convenient 8-hourly schedule and to facilitate recording of the clinical events. Administration of drugs for five days and nutrients will be done in random double blind design to 4 cells in a dose of 2 times of the RDA for a period of 2 weeks.

Formulation of syrup in different groups:

Group A: Zinc + M (zinc daily dose 20 mg elemental)
Group B:Calcium + MV (calcium daily dose 800 mg)
Group C:Zinc+ Calcium+ MV
Group D:MV

Growth Study:

At the end of clinical study at hospital, patients will be followed up at home at 2 weekly interval upto 3 months for their growth. Weight, height and Mid upper arm circumference will be measured with appropriately trained research officer.

Determination of Nitrate in serum

Serum will be stored at 20^0 c until analysis . The sum of nitrate and nitrate in serum will be measured by a colorimetric method based on the conservation of nitrate with the use of nitrate reductase from Aspergillus. For the assay 0.1 ml of serum will be diluted with 0.5 ml 0.035 M phosphate buffer, pH 7.5. After addition of 0.05 ml nitrate reductase (I mu/i) and 0.05 ml of nicotinamide adenine dinucleotide phosphate, reduced (NADPH) (1.8mm), the reaction mixture will be incubated at room temperature for 2h. Excess NADPH will then be oxidized by adding 0.05 ml phenazine methosulphate (8 nm), and the mixture will be deprotinized with 0.l ml zinc acetate (0.5) and 0.1 ml NaoH (0.5). followed by centrifugation at 3000 rpm for 10 min. The clear supernatant will be used for the Griess assay of nitrate by adding 0.25 ml sulfanilamide (0.1 M im 1.5 M phosphoric acid) and 0.25 ml naphthylethy enediamine (8 mM). The resultant colour will be read out in a colorimeter at 540 nm. Same procedure will be done for the determination of nitrate in urine.

Nitrate will be determined in physiology laboratory of Clinical sciences division.

Lipid peroxidation:

This laboratory has also established an efficient method to assess TBARS (Khaled et al.), an index of lipid peroxidation (LPO). As mentioned earlier ROS activity is usually assessed by measuring LPO in terms TBARS. TBARS will therefore be estimated in our laboratory using the already selected method.

Data Analysis

After the completion of the study, coded data will be entered into an IBM compatible diskette and statistical analyses will be carried out by SPSS software package. For comparing the categorical data Chi-square or Fisher exact test will be performed and for quantitative data student's `t' analysis of variance test or relevant non-parametric test will be done. The four groups will be compared according to admission characteristics to determine their comparability. Impact of micronutrients will be compared under the following parameters:

<u>Variables on which data to be collected</u>:

Clinical:

1. Cumulative stool frequency on day 3 and day 6 will be compared between two groups.

2. Duration of blood in stool.

2. Duration of blood in stool.
3. Duration of abdominal pain and tenesmus.
4. Duration of fever (>37°C).
5. Feeling of well being (Patient's/Physician's record by inquiry).
6. Improvement of appetite (Patient's opinion).
7. Increased growth.
8. Changes of body composition at day-1,day-15 and day-90.

Laboratory:

1. No. of faecal leucocyte and RBC in the stool on admission, day 3 and day6
2. Duration of excretion of organism (Shigella spp.) in the stool.
3. Estimation of stool protein loss on admission, day- 3, day-5, day-15 and day-90 by alpha-1 antitrypsin assay.
4. Estimation of serum zinc and calcium on admission, day-15
5. Lipid peroxidation on admission,day-15.
6. 3-Methyl histidine excretion in urine (indicator or protein breakdown from muscles).
7. Test of immunity before and after supplementation of micronutrients by estimation of IL-1, IL-6 on Day 1, day-5 and day-15
8. Multiple antigen test for CMI on admission and at Day 30.
9. Estimation of nitric oxide production through mesurement of nitrite and nitrate in urine and serum on admission, day-5, day-15 and day-30.

Rationale of the Study

Shigellosis has been recognized as an important cause of morbidity and mortality in the developing countries. Both diarrhoea and malnutrition are common in the developing countries. Zinc deficiency may be possible factor for increasing diarrhoeal incidence or prevalence. However, the role of zinc and calcium on severity of disease and subsequent growth in shigellosis has not been adequately evaluated. In malnourished children, there is a significant depression of immunity. In animal study it was observed that low zinc concentration decrease the number of helper T-cells and increase the suppression activity of immune response. Moreover, malnourished children are more prone to oxidative damage. It was demonstrated in animal study that hypoprotienemia has also been implicated to aggravate lipid peroxidation. In shigellosis hypoproteinemia bacterial overgrowth, mucosal injury or malabsorption of nutrients may lead to the deficiency of both macro and micronutrients. The importance of micronutrients have recently been recognized in the treatment of many intestinal diseases. However, very little is known about the therapeutic role of micronutrients in protein loosing enteropathy including shigellosis. The results of the study may indicate improved therapeutic outline for severe disease like shigellosis.

Protein, calmodulin, containing Ca is associated with NO production during macrophageal/phagocytic activity. Over production of NO creates a noxious oxidative process which of course does not require Ca. Zn, on the other hand, in addition to all the immunological activities is also an important component of super oxide dismantoze (SOD), an endogenous antioxidant. Study of antioxidative activity of Zn and Ca would be an important expect of this study in order to understand their role as antioxidation nutrients.

Working definitions of outcome variables:

The treatment effects will be assessed by changes in the following variables.

1. **Duration of dysenteric illness**: The duration of illness will be determined by the time interval from the start of treatment to the end of diarrhoea.

2. **The end of diarrhoea:** The end of diarrhoea will be defined when there will be 2 or less formed stool during any 24 hour preceding period.

3. **Dysenteric stool**: This is defined as thick liquid stool having mucus and/or blood that takes the shape of the container and can be poured easily from the container. Clearance of mucus and blood from the dysenteric stool will be checked by inspection daily.

4. **Soft stool**: This type of stool is the characteristic of early improvement of the dysenteric illness. It is more thick, may have mucus, takes the shape of the container but can not be poured easily.

5. **Formed stool**: This is the normal type of stool and is characterized by the absence of mucus and blood, it retains its own shape, and does not stick to the container.

6. **Abdominal pain, tenesmus, and rectal prolapse:** These symptoms are very characteristic of acute shigellosis and changes in these symptoms would reflect the effects of any treatment. Pain may be present in the upper or lower abdomen, may be constant or periodic, and may vary in severity.

For the purpose of evaluation, these will be categorized as mild, moderate or severe types. Instances of rectal prolapse are common in children but rarely found in adults with shigellosis. Prolapse if present, will be defined as complete, partial, or reducible.

Definition of treatment failure:

If the patients' condition deteriorates in spite of 3 days drug therapy, then they will be considered as treatment failures. These patients will be treated according to the standard treatment of hospital.

Complications: Development of HUS, toxic colitis, enchephalopathy, Septicaemia

Management of dropout cases in the study:

1. If the patient leaves the study after randomization and before completion, the data will be excluded from the analysis and the position will be replaced by a new entry at the end of the randomization list.

2. If a patient develops clinical complications (i.e. severe hyponatremia, leukemoid reaction, HUS, septicaemia, intestinal obstruction, paralytic ileus etc) which prevent continuation of treatment with zinc and calcium, the patient will be withdrawn from the study, and the position will be replaced with a new entry.

Time frame for the study

Month 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Activity
Organization------
Training of staff-------
Recruitment --
Lab test --
Data collection ____
Data analysis ----------------------
Report writing ------------
Dissemination ---------
Quality Control
of data collection --

SUMMARY OF PROCEDURES

On admission (Day-1).

1. Stool microscopy for RBC's pus cell and parasites and stool culture for Shigella spp.
2. Blood for total and differential white blood cell counts, microhaematocrit (routine).
3. Serum electrolytes (Na, K, Cl, TCO_2, Creatinine)
4. Stool for alpha-1 antitrypsin
5. Weight & Height,MUAC
6. Estimation of serum zinc,copper and calcium.
7. Lipid peroxidation (blood & stool).
8. Test of immunity for CMI & IL-1, IL-6
9. 3-Methyl histidine excretion in urine
10. Estimation of serum nitrate and nitrites
11. Estimation of Urinary nitrate and nitrite

Day-2.

1. Summarize clinical findings and number of stools and their character on an 8 hourly basis.
2. Stool and rectal swab culture for Shigella spp.

Day-3.

1. Summarize clinical findings and number of stools and their character on an 8 hourly basis.
2. Stool and rectal swab culture for Shigella spp.
3. Serum electrolytes.

Day-4.

1. Summarize clinical findings and number of stools and their character on an 8 hourly basis.
2. Stool and rectal swab culture for Shigella spp.
3. Serum electrolytes.

Day-5.

1. Summarize clinical findings and number of stools and their character on an 8 hourly basis.
2. Stool and rectal swab culture for Shigella spp.
3. Lipid peroxidation (blood & stool),
4. IL-1, IL-6,
5. Nitrate and nitrites in blood and urine
6. Stool α1-antitrypsin
7. 3-methyl histidine excretion in urine

Day-15

1. Summarize clinical findings and number of stools and their character
2. Weight & Height,MUAC
3. Estimation of serum zinc and calcium.
4. Lipid peroxidation (blood & stool)
5. 3-methyl histidine excretion in urine
6. Urinary nitrate and nitrite
7. IL-1 and IL-6

Day-30

1. Test of immunity for CMI,
2. Wt. Ht, MUAC

Day-45 Wt, Ht, MUAC

Day-60 Wt, Ht, MUAC

Day-75 Wt,Ht, MUAC

Day-90

1. Summarize clinical findings and number of stools and their character
2. Weight & Height
3. Estimation of serum zinc and calcium.
4. Lipid peroxidation (blood & stool)

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ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

In this protocol we are proposing a blind, controlled, clinical trial of zinc and calcium in the treatment of patients with acute shigellosis. These micronutrient and nutrients have no known side effects in man in present doses. There is a strong proposition that the study groups will be benefited with the supplements.

In this trial 172 patients (age between 1 to 3 yrs) with acute shigellosis will be studied. Micronutrients will be given orally (at a daily dose Zinc=20 mg elemental, Calcium = 800 mg) in children. All patients will be treated with Pivmecillinum as the standard treatment for shigellosis. Clinical data including body weight, height, duration of illness, stool volume, rectal swabs will be collected during the study. The patients will be admitted for 5 days in the hospital and 2 ml blood samples will be taken on admission, day 5 and day 15 for electrolyte and blood counts and immunity tests.

Parents of all patients will give their informed consents before admitting them into the study. For patients, consents will be obtained from their parents or guardians. All patients will have the right to enter or leave the study at any point of the trial.

Patients will be given standard treatment and hospital care as indicated by their clinical conditions.

All clinical information will be regarded as confidential and the final report will be published without reference to patients' name or identity.

Answer to reviewers comments to the protocol entitled

"Impact of supplementation of micronutrients in shigellosis in children"

Reviewer no 1.

Comment 1.

Reviewer supports the hypothesis and sees as a potentially useful study, and views the collaborative effort to likely to acheive its goal

Answer: Thanks for agreement

Comment 2.

The authors could give more details to justify why the particular micronutrients have been selected.

Answer: Zinc, copper and calcium have been selected as relevant micronutrients as to add to the better clinical recovery through improved immunity, mucosal repair, and better protein synthesis during acute infection and subsequent period. This has been elaborately justified by the back ground Information. There are potentials in children but this study will explore whether there is any benefit in children with shigellosis.

The reviewer also says, that the proposal does not make clear the relationship between these specific micronutrients and lipid peroxidation.

Answer: Because lipid peroxidation is well recognized pathophysiological process during cellular damage, no specific information is available as to the antioxidant effect of zinc, copper, calcium in shigellosis. This will be measured in this study to explore their role. Calcium containing protein calmodulin is associated with nitric oxide (NO) production by cNOS (constitutive nitric oxide synthase). Excess NO production is also harmful due to its free radical activity. In addition, copper, zinc and calcium are associated with the Supper Oxide of Dismutase (SOD) which is an endogenous antioxidant. In addition,Cu is also a core trace element in the protein called ceruloplasmin which is also another strong endogenous antioxidant. Benificial effect of copper, zinc and calcium are therefore expected where there is a possibility of deficiencies of these trace elements.

Methodology:

Comment 1.

The study may recruit children who are not deficient in micronutrients.

Answer: The children will be moderately malnourished i.e 61-75% wt/age, and likely to have a fair degree of deficiency in micronutrients. The study will look into clinical and host response to micronutrient supplementation, this will be tested in shigella patients who are right kind of study subjects. 48 hours has been taken as a cut off point for acute illness to study the clinical effect of supplementation.

Comment 2:

Why did the authors not choose to include a further study group in which all the micronutrients under study were given to the patients in addition to Pivmecillinum?

Answer:

The combined effect may be tested. But there may be problem of micronutrient interaction that inhibits absorption of other micronutrients. The second is results will not indicate which micronutrient was beneficial for which purpose and which one is comparatively better and by how much difference. To clarify that, a factorial design would be needed and study would involve more groups and subjects and result much increase in cost. Fund is of considerable importance.

Comment: Budget to refine for administrative cost.

Answer:

Minimum budget has been presented, It would be possible to adjust if we get specific suggestions.

Answer to comments of the Reviewer no 2.

Comments: Copper supplementation may increase generation of free radicals.

Answer: It is known that excess coppper involve in the generation of 'OH" ion through the Fenton reaction. However,Cu and Zn both are associated with the Supper Oxide of Dismutase (SOD) which is an endogenous antioxidant. In addition,Cu is also a core trace element in the protein called ceruloplasmin which is also another strong endogenous antioxidant. Benificial effect of copper, zinc and calcium are therefore expected where there is a possibility of deficiencies of these trace elements.

Comments: IL-2 and IL4, and IL8/IL-6 ratio will be better for estimating inflammatory response in shigellosis.

Answer: We are measuring IL1 which will give precise indication of inflammatory condition of epithelial damage leading to severity of shigellosis. Additional benefit by further interleukin assay is less likely and more expensive.

Besides, we will see lipid peroxidation both in blood and stool to see the oxidative stress as a severity of infection.

4.2 BUDGET ESTIMATES FOR SUPPORT REQUESTED - contd

Specification

*1 Personnel

A. Professional Scientific Staff

Names	% time	# months	1st year US$	2nd year
Dr. S. K. Roy	15%	12	3,140	3,360
Dr. G. Fuchs	10%	12	-	
Dr. M. A. Khaled	Consultant			
Dr. T. Azim	10%	12	1,270	1,360
Mrs. S. Islam	10%	12	510	546
Dr. A. M. Khan	15%	12	1,953	2,090
Sub total			6,873	7,356

B. Technical Staff

Research Physician	100%	12	3,000	3,000
Research Officer	100%	12	4,476	4,790
Senior Health Assistant (2)	100%	12	6,888	7,370
Female Volunteer (3)	100%	12	1,250	1,250
Secretarial Service	10%	12	1,000	1,000
			16,614	17,410

Consultant cost	5,000	-------

* 2 Equipments (no. X unit cost)

Weighing scales	2 X $ 600=	1,200	-
Length Board	4 X $ 150=	600	-
Microcomputer	1 X $ 3,000=	3,000	-
Sub-total		4,800	

3 Supplies		1st year	2nd year
Medical supply		500	500
Office supply		1,000	1,000
Lab supply		1,000	1,000
Biochemical test	No. assays X unit cost		
Zinc	350 X $ 6	1,100	1,000
Calcium	350 X $ 6	1,100	1,000
Lipid peroxidation test	350 X $ 10	2,000	1,500
Immunology test			
CMI kits	350 X $ 9	1,600	1,500
IL-1	350 X $ 50	9,000	8,500
IL-6	350 X $ 30	5,500	5,000
Sub Total		22,800	21,000
Publications		200	900
Other expenses *4			
Total drugs		1,000	-
Pathology tests		1,000	1,000
Patient Hospitalization		9,000	8,000
Patient follow up		1,300	1,200
Air freight, communication		1,300	500
Fax, DHL, Email		500	500
Paper, photocopy		300	300
Stationeries		250	200
Sub total		14,650	11,700
Total direct cost		70,937	58,416
31% overhead cost		21,990	18,108
Total Project Cost		92,927	76,524

Total for entire project period **US $ 1** **169,451**

Jesmin
04-09-96