Attachment 1.
(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 11/12/91

Principal Investigator Dr Shahadat Hossain

Trainee Investigator (if any) _____

Application No. 91-017

Supporting Agency (if Non-ICDDR,B) _____

Title of Study "Vitamin A supplementation in the treatment of shigellosis in children"

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.

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

Hossain
Principal Investigator

Trainee

91-017
11/12/91

RESEARCH PROTOCOL

1. TITLE: VITAMIN A SUPPLEMENTATION IN THE TREATMENT OF SHIGELLOSIS IN CHILDREN

2. PRINCIPAL INVESTIGATOR: Dr. Md. Shahadat Hossain

   CO-INVESTIGATOR: Dr. S.A. Sarker

   PROJECT COORDINATOR: Dr. D. Mahalanabis

   CONSULTANT: Dr. D. Habte

3. STARTING DATE: January 1992

4. COMPLETION DATE: 3 years from starting date

5. TOTAL DIRECT COST: US$ 225,112

   POSSIBLE SOURCE OF FUNDING: US AID

6. SCIENTIFIC DIVISION: This protocol has been approved by the Clinical Sciences Division

Signature of Associate Director, CSD --Kabir--

Date: --11-12-91--

ABSTRACT SUMMARY

This study involves the investigation of the impact of single large dose of Vit A 200,000 unit on acute shigellosis in children. The study is a randomized double blind clinical trial. 230 children between the age 1-7 years with acute shigellosis will be divided into two groups; one group receiving vitamin A therapy along with antibiotics and the other group will receive antibiotic only. The severity and duration of the illness will be recorded and compared among the two groups.

The study design will ensure that children who are not receiving vitamin A at study entry will receive vitamin A supplementation at study exit for ethical reasons. Serum vit A levels pre-and 5 hours post treatment will be measured to evaluate the adequacy of absorption during acute shigellosis.

8. REVIEWS:

(a) Ethical Review Committee: ----------

(b) Research Review Committee: ----------

(c) Director, ICDDR,B:----------

RESEARCH PLAN

A. INTRODUCTION

1. Objective

The objective of this study is to evaluate the effectiveness of vitamin A in the treatment of shigellosis.

2. Background

a) Diarrhoea is a leading cause of morbidity and mortality among children in Bangladesh and other developing countries. In rural Bangladesh diarrhoea is responsible for 15% of infant mortality and over 50% of mortality in children 1-4 years of age (1).

Vitamin A deficiency is also an important health problem in much of the developing world including Bangladesh (2). The estimated prevalence of vitamin A deficiency in Bangladeshi children, including nightblindness, bitot spots and active corneal involvement, far exceeds WHO criteria for the definition of a major public health problem (3). An estimated 30,000 Bangladeshi pre-school children are blinded each year (3) and the majority of these cases are attributed to vitamin A deficiency. Globally it is estimated that 500,000 children annually develop sight threatening lesions (4).

Groups at increased risk include pre-schoolers, particularly those older than two year (3) male children, nonbreast-fed (3, 2) and children with respiratory or diarrhoeal illness (3, 5).

Clearly, both diarrhoeal disease and vitamin A deficiency are responsible for a large proportion of the childhood morbidity and mortality in Bangladesh and much of the developing world.

The increasing evidence of a relationship between vitamin A deficiency and infection has prompted interest in the possible therapeutic benefits of vitamin A supplementation for clinical illnesses such as diarrhoea. The Control of Diarrhoeal Disease (CDD) Programme of the World Health Organization has identified the question of therapeutic benefits of vitamin A for diarrhoea as a research priority. The proposed study will address the question of the effectiveness of vitamin A for treating invasive diarrhoeal episodes and provide further information regarding the relationship of these two conditions.

b) Animal studies indicate that vitamin A is important for normal growth, and resistance to infection (6). Numerous studies in a variety of animal species demonstrate that both humoral and cell mediated immunity are compromised (7).

Epidemiologic studies in a variety of settings clearly demonstrate a relationship between vitamin A deficiency and infection. In Bangladeshi children diarrhoea and measles have been found in association with xerophthalmia (8). Depressed serum vitamin A levels were detected in Guatemalan adults

and children suffering from respiratory and diarrhoeal infections (9). In the Philippines, children with mild xerophthalmia presented more frequently than controls with respiratory disease but did not differ from controls in the incidence of diarrhoea (5). Studies of Indonesian children have demonstrated that those with mild xerophthalmia have a mortality rate 4-12 times that of clinically normal children (10). In this same study surviving children with pre-existing mild deficiency had a significantly increased risk of developing respiratory or diarrhoeal illness (11). This finding supports the hypothesis that vitamin A deficiency predisposes to infection.

There is a conflicting data regarding the benefits of vitamin A supplementation for infection. In a study of vitamin A supplementation in Indonesian villages, there was a 30% reduction in childhood mortality in villages receiving vitamin A (12). In this study the prevalence of xerophthalmia, which was used to estimate the prevalence of vitamin A deficiency was only 5%. This suggests that many children without clinical signs of vitamin A deficiency may still be deficient and predisposed to infection. In contrast, it is found that large doses of vitamin A failed to enhance antibody response to tetanus toxoid in Bangladeshi children (13). However, in this study the presence of infection was not controlled for and infection may affect the ability to mount an immune response.

(c) Considering the biologic rationale there are two possible mechanisms by which vitamin A deficiency may aggravate invasive diarrhoea.

1. Role of vitamin A at the local level: vitamin A is necessary for maintaining the integrity of the mucus-secreting epithelial tissues that serve as a mechanical barrier against infection (14, 15, 16). Deficiency of vitamin A leads to loss of protection against colonization of invasive organisms.

Mild vitamin A deficiency has been found to be associated with abnormal cellular kinetics in the small intestine of rats (17). In this study the duration of cell cycle was lengthened mainly due to lengthing of DNA synthesis. There was also impaired migration of cells out of crypts. In another study of local effects of vitamin A demonstrated a decrease in the number of Goblet cells in the small intestinal mucosa (18). Vitamin A was necessary for the biosynthesis of Goblet protein (14). These findings suggest vitamin A plays a role in regulation of cell division in the small intestine and is necessary for the production of normal cell glycoprotein. Since replacement of the affected cells is the key to recovery from toxin induced diarrhoea, vitamin A may accelerate the regeneration time of normal intestinal epithelium by restoring normal cell kinetics and enhancing the production of normal cell glycoprotein, thereby shortening the duration of diarrhoeal illness and contributing to a decreased stool output. Clearly these same properties could effect recovery of the structural integrity of the intestinal epithelium in diarrhoea due to an invasive organisms as well.

The intestinal mucosa has a fast turnover of 36 hours, and this may contribute to the ability of vitamin A to have an impact within a short-time. The intestine is a very vascular organ and this property may facilitate the rate of delivery of vitamin A to the tissue since serum levels of vitamin A and retinol binding protein rise within 4 hour of vitamin A supplementation (19). Vitamin A is quickly available to facilitate the healing process. Adequate absorption of vitamin A during diarrhoeal illness has documented in

three studies (19, 20, 21). In summary, the evidence suggests that it is theoretically feasible for vitamin A therapy to have an impact on diarrhoeal duration and severity during an acute episode.

2. Role of vitamin A in immune system function.

The interactions between nutrition, immunity and infection have been the focus of much recent work. It has been observed that protein energy malnutrition (PEM) is associated with impaired immuno competence (22, 23, 24), including depressed cell-mediated immunity. Phagocyte dysfunction, decreased levels of complement components, reduced mucosal secretory antibody responses and lower antibody affinity.

It was observed in one study that the overall mortality rate in victims of PEM was 15%, whereas it was as high as 80% if malnutrition was associated xerophthalmia (25). The association between hypovitamonosis A and bacterial and viral infection in humans and various species of animals has been reviewed (26, 27, 28). Many pathological mechanisms probably contribute to the increased risk of infection in vitamin A deficiency including tissue changes and altered specific and nonspecific immunity. Furthermore, infection itself can aggrieve vitamin A deficiency.

Mucosal immunity:

Secretory antibody response, mucosal cell-mediated immunity and nonspecific functions are important host defences. The disastrous effect of infection in vitamin A deficiency state may be due to the increased pathogenecity of infection agents and/or reduced immunocompetence of the host. Hypovitamonosis impairs tissue integrity by permitting keratinization of mucocilliary linings. In the intestine, a reduction in the number of Goblet cells and in mucus production disrupts nonspecific defense mechanisms of gastrointestinal tract. Impaired barrier function increases the systemic spread of infectious agents. By exposing vitamin A deficient rats to the nematode *Angiostrongylus cantonensis*, it was shown that the penetration power of the larvae increased and the animals had a higher worm burden and shorter survival period (29).

Increased keratinization, low levels of mucus secretion, and decreased number of Goblet cells in the intestine in vitamin A deficiency together with reduced SIgA secretion and reduced local antibody response, act synergistically to make the deficient individual vulnerable to a variety of gastrointestinal and respiratory infections. In addition, because of compromised immiunocompetence, such an individual may succumb to repeated and chronic infections (30, 31).

Phagocytes:

Once the infectious agents crosses the anatomic barriers of skin and mucous membrane and enters the body, phagocytes deal in a nonspecific way with certain bacteria and fungi before antigen-specific cellular and humoral immune mechanisms come into play. Large doses of vitamin A given to normal mice have been shown to offer nonspecific resistance to gm +ve and gm -ve bacterial and fungal infection (32). A decreased mortality rate and low levels of bacteraemia were reported after challenge with Pseudomonas aerunginosa, Listeria monocytogenes and Candida albicans.

Lymphoid tissue:

A diet deficient in vitamin A has been shown to cause atrophic changes in the thymus and spleen in rats (33). Pair-fed rats that developed PEM also showed atrophic changes in both tissues, but the magnitude of change was much greater in the vitamin A deficient group.

Adjuvant effects:

Vitamin A acts as an adjuvant at nontoxic doses and enhances cell-mediated and humoral immune responses. Injections of vitamin A increased the cellularity of regional lymphnodes (34). The vitamin has also been shown to stimulate antibody production to bovine gamma globulin, which would otherwise have resulted in immunological paralysis (35). The adjuvant property of vitamin A was thought to be due to its membrane-labilizing effect on lysosomes. Lysosomal membrane labilization can induce lymphoid cell proliferation (36). Vitamin A has been shown to enhance cell-mediated immune response when administered simultaneously with or close to the antigen challenge.

Thus it is reasonable to expect that vitamin A deficiency is associated with an increased incidence of infection and that its prevention or treatment will result in decreased illness. This may be due to epithelial changes and improved cell-mediated and mucosal immiunity. Recent works (30, 31; 37-40) has affirmed the critical role of beta-carotene and vitamin A in optimum immunocompetence. At the same time, massive doses of vitamin A if given for prolonged periods may have a deleterious effect.

Possibly there is a bi-directional relationship between infection and vitamin A deficiency, similar to the relationshiop of protein calorie deficiency and infection (figure 1). Diarrhoea may lead to decreased absorption of vitamin A and associated anorexia could cause decreased intake. Deficiency in turn leads to increased susceptibility to infection.

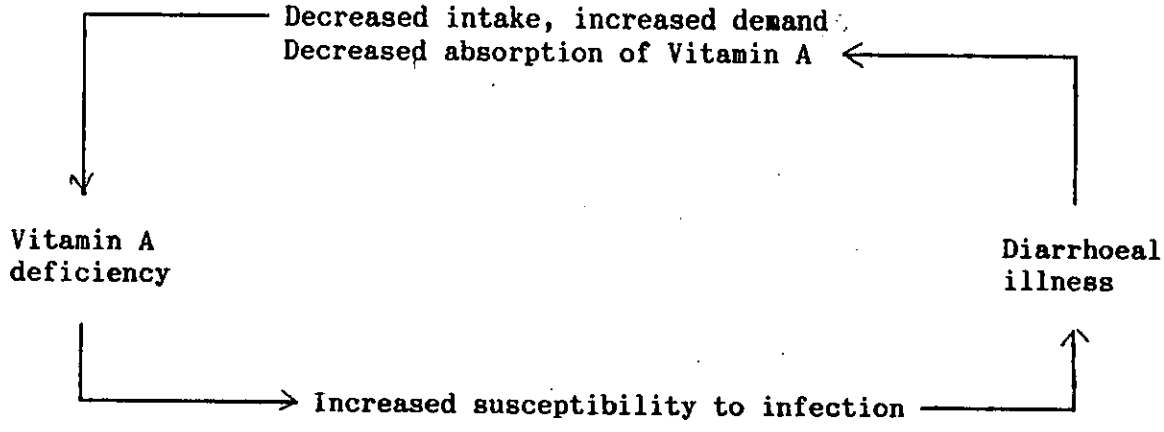

Fig. 1. Interaction of Vit. A deficiency + diarrhoea.

3. Rationale

The ICDDR,B has identified the question of therapeutic benefits of vitamin A for dysentery as a research priority. The proposed study will address the question of the therapeutic effectiveness of vitamin A for invasive diarrhoea especially for shigellosis and provide further information regarding the relationship of these two conditions.

B. SPECIFIC AIMS

To determine whether a large single dose of vitamin A (200000 I.U.) reduces the duration and severity of symptoms of the patients suffering from acute shigellosis, with subclinical vitamin A deficiency being common in the population from which patients are derived.

C) Experimental design and methodology:

This is a randomized double-masked clinical trial using vitamin A to treat children suffering from dysentery.

Randomization reduces bias due to patient variables that may affect outcome. Outcome can be assessed more accurately in a controlling clinical setting. The basic study design is outlined below:

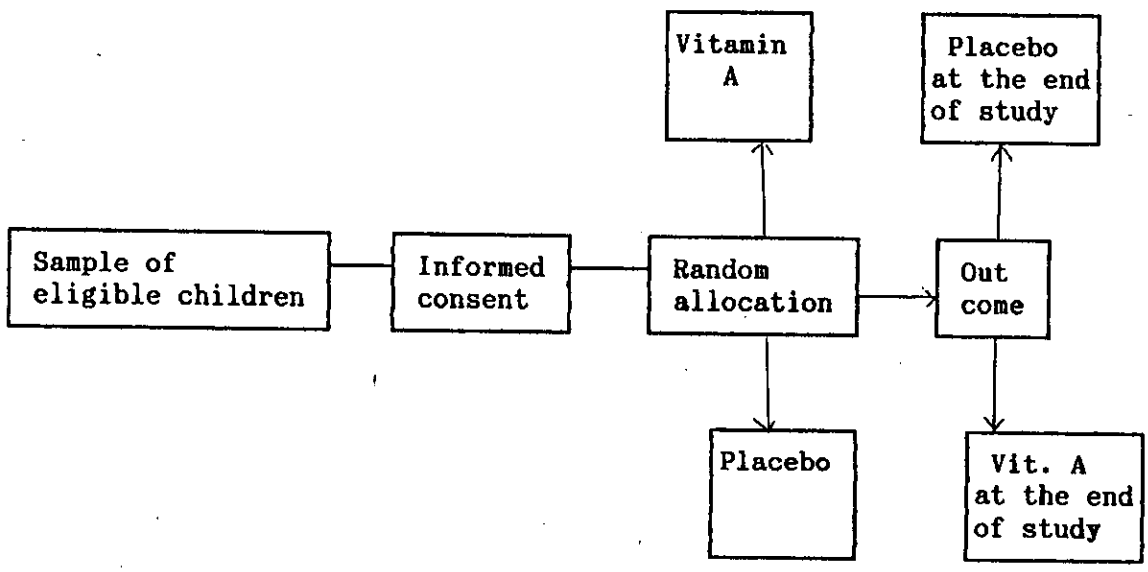

Fig. 2. Study design.

Setting:

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has a treatment centre at Dhaka where approximately 60,000 cases of diarrhoeal patients were treated in the year 1990 out of which about 13% had dysentery. The Centre facilities are designed specifically for the investigation and treatment of dysenteric illness and clinical staff are familiar with this work. Study children will be admitted to the study ward where principal investigator, co-principal investigator and trained personnel

will collet the data for the research and also will be responsible for their management.

Patient selection:

Children aged between 1-7 years who are suffering from uncomplicated dysentery of less than 72 hours duration will be considered for entry into the study. Only children without signs and symptoms of vitamin A deficiency will be eligible for study enrollment (appendix 1).

Dysentery will be defined as three or more stool occurring within the last 24 hours associated with blood with or without mucus in the stool.

The above criterion will identify children with a clinical presentation compatible with shigellosis.

Exclusion criteria:

1. Age less than one year or greater than 7 year.

2. Dysentery longer than 72 hours before hospitalization.

3. Coexisting infections such as pneumonia, meningitis or UTI diagnosed by the attending clinician at initial examination.

4. History of nightblindness or clinical sign(s) of vitamin A deficiency as determined by attending clinician according to criteria in Appendix 1.

5. Clinically marasmic and kwashiorkor patients.

6. Vitamin A supplementation:

(1) Daily supplementation (very uncommon).

(2) 200,000 I.U. (as part of mass dosing programme) received in the past 90 days. These children are excluded since the protective effects of vitamin A are thought to last approximately 60-90 days.

Informed consent will be obtained by reading of the informed consent form to the parents of potential study subjects (Appendix 2).

Sample size calculation:

1. Clinical improvement on day 3 (assessment by two experienced clinicians): Based on a recent trial of antibiotics in children we expect marked improved by day 3 in 57% of the patients (Alam AN et al. — unpublished). This assessment was carried out blindly and was based on clinical picture, stool character and general wellbeing of the patients. Assuming on improvement of 75% in the group receiving vitamin A the sample size (at 5% significance and 80% power) is 107 in each group; with some dropouts we propose a sample size of 115 in each group i.e. 230 total.

2. Cumulative stool frequency for first 3 days: Based on a recent clinical trial the cumulative stool frequency (± SD) in the group treated with the

standard first line drug (nalidixic acid) was 46 [(± 14) (Alam AN et al. — unpublished)]. We expect it to be reduced by 20% (as has happened in the group with a new antibiotic); the calculated sample size (5% significance, 80% power) is 37 in each group; with dropouts 40 in each group is proposed, i.e. 80 in total.

Taking the larger of the two we propose a sample size of 230 (total). The sample size will again be calculated by an independent person after enrolling 35% of the sample and adjusted accordingly.

Randomization procedure:

Randomization will be carried out by a person not directly involved in patient care. Permuted blocks of random number will be used to assign children to study or control group. The vitamin A syrup and simple syrup will be identical in appearance, taste, and flavour. Each patient will have a pair of bottles assigned to him/her, one containing 200,000 units of Vit A in syrup and the other containing the syrup without vitamin A. For the study patient the first dose will be Vit A syrup and the second dose simple syrup. Pairs of bottles will be serially numbered to correspond to the serial number of the patient enrolled. According to randomisation code the Vit A syrup bottle on the pair will be lebelled as the 1st dose and simple syrup bottle as second dose for patients assigned to the study group. For the patients in the control group the order of the pair of bottle is reversed.

Treatment protocol:

After children are enrolled in the study, they will be assigned to receive treatment from the bottle marked 1st dose. Both the staff and patient will be masked as to the content. A medicine dropper will be used to dispense the dosage into the child's mouth. The treatment group will receive 200,000 I.U. of vitamin A at the time of admission. Children who vomit within two hours of admission will be given a further 200,000 units. The rationale for this dosage is two fold:

(i) This dosage has been used safely in previous studies in this age group in children with infection (19, 20) and is also the currently recommended dose for mass prophylaxis.

(ii) This dosage is currently recommended to treat xerophthalmia. The intestinal mucosa may respond to a similar regime.

On admission a rectal swab will be taken and sent for culture, stool will be sent for microscopic examination and after proper hydration blood will be drawn to measure serum vitamin A, pre-albumin, retinol binding protein, serum electrolytes, creatinine, C-reactive protein and for complete blood count and platelet count. The sample of Vit A, pre-albumin, RBP, CRP will be assayed with standard techniques and the results will be held by a biochemist of nutrition biochemistry laboratory not directly involved with this study and will be kept masked till completion of the study. Subjects will then receive antibiotic therapy and the study outcome measures will be assessed and recorded as outlined below.

Definition of outcome and method of measuring:

Outcome variables have been chosen according to what vitamin A is most likely to affect. The outcomes and their measurement are standardized so that they may easily be compared to other diarrhoea morbidity studies.

Duration of illness and stool frequency and presence of blood and mucus or blood or mucus in the stool are the outcomes of primary interest. Secondary outcomes such as fever, intake and urine output are of less importance but are routinely recorded so this data will also be included. Vomiting is a symptom of vitamin A toxicity so it will be important to monitor this as well. Stool microscopic examination and rectal swab culture will be done routinely.

Measure of illness duration:

We will evaluate both the clinical and bacteriological response to therapy. Clinical response will be judged on the basis of the patients day 3 and day 5 of hospitalization and will be graded as follows:

Measurements of outcome variables.

Clinical

1. Cummulative stool frequency on day 3 and day 5 will be compared between two groups.

2. Duration of blood in the stool.

3. Duration of fever (>37°C).

4. Duration of abdominal pain and tenderness.

5. Feeling of improvement/well being (mother's/physician's impression).

6. Improvement of appetite (mother's impression).

Laboratory

1. No. of faecal leucocytes and RBC in the stool.

2. Duration (in days) of excretion of organisms (*Shigella spp.*) in the stool.

3. Comparison of C-reactive protein on Day 1, Day 3, and Day 5.

4. Comparison of stool protein loss on Day 1 and Day 5 (by α_1 antitrypsin).

Measurement of other variables:

Serum levels. Vitamin A, pre-albumin and retinol binding protein levels will be measured pre-treatment and 5 hour post-treatment. These measurements are needed in order to assess the baseline levels and to confirm adequate absorption of vitamin A.

Side effects/toxicity. Side effects are unlikely to occur. This dose has been used safely in this age group in many mass supplementation programmes (41). Long term intake of 10-20 times the recommended dosage can lead to signs and symptoms including nausea, vomiting, headache and skeletal pain.

Baseline examination:

A baseline history and examination will be obtained to determine the subjects eligibility for inclusion in the study and to collect relevant data prior to beginning the study that will allow:

1. Comparison of the study group after randomization, and

2. description of the study population to determine whether the results obtained can be compared with those from other trials.

The baseline history and examination will include identification of patients, a description of symptoms prior to admission and the duration, details of any treatment given for the illness, a description of the feeding status prior to admission, a description of the stool prior to admission, results of the physical examination including the state of hydration and nutrition, and results of stool microscopy which is a part of inclusion criteria. The above information will be recorded on pre-designed and pre-tested forms.

Informed consent:

If the patient is found to be eligible for inclusion into the study an informed consent will be obtained.

Allocation to treatment groups:

After enrollment in the study the subjects will be randomly allocated to treatment groups according to the method described; randomisation is coded in the in the serially numbered paired bottles.

Case management:

Treatment will commence after obtaining baseline data which will include history, physical examination, stool and rectal swab for culture, stool microscopy, admission blood sample, weight and height. Patients will be treated with ORS if he/she has signs of dehydration. Food will be offered appropriate for age at regular intervals. Antibiotic (Nalidixic acid 55 mg/kg/d in four divided doses will be given orally). Subsequently, if a case is found to be resistant to Nalidixic acid, they will receive appropriate drug as per sensitivity report.

Withdrawals from the study:

If a patient leaves the hospital before the end of the study, data up to the point of leaving will be considered in the analysis. If a patient develops complication which prevents the planned treatment to continue the patient will be withdrawn from the study e.g. HUS (who may require transfer to another hospital), paralytic illeus, septicaemia, pneumonia, meningitis etc.

Data up to the limit of withdrawal will be considered in the analysis. Records will be kept of the subsequent course of illness and final outcome.

Organization of the trial:

Patients will be selected from those attending out-patient and admitted to study ward if they fulfill inclusion criteria. The PIs with the assistance of a full time medical officer will take care of the patient. Eight-hourly evaluation will be recorded on a predesigned form. Patients will be admitted from those seen in the morning up to 11 a.m. to enable a convenient 8-hourly schedule and facilitate recording of relevant events.

Number of stools will be recorded using open diaper. Blood in the stool will be noted as they occur on a tally sheet and summarised.

Summary of procedures:

On admission (D_1)

1. Stool microscopy for RBC's pus cells and parasites and stool culture for shigella species, Salmonella spp., Campylobacter spp., V. cholera, plesimonas shigelloides, and A. hydrophila.

2. Rectal swab culture for shigella species.

3. Blood for total and differential white blood cell counts, microhaematocrit (routine).

4. Serum vitamin A, pre-albumin and retinol binding protein, serum electrolytes, creatine, SGPT, c-reactive protein.

   Serum vitamin A, pre-albumin and retinol binding protein will be repeated 5 hour post-treatment.

5. Stool for α_1 antitrypsin.

Day 2

1. Summarize clinical findings and number of stools and their character on an 8 hourly basis. Each stool will be characterized.

2. Rectal swab and stool culture for shigella.

3. Stool for M/E.

Day 3

1. Summarize clinical features, number of stools and their character on 8 hourly basis. For characterization of stool, standard methods will be used like watery/mucoid/mucoid + blood etc.

2. Rectal swab and stool culture for shigella.

3. Blood sample for vitamin A level, pre-albumin and retinol binding protein, microhaematocrit, serum elecltrolytes, c-reactive protein.

4. Stool for M/E.

5. Record summary response.

Day 4

1. Same as under day 3 except for blood sample.

Day 5

1. Same as under day 3 except serum electrolytes.

2. Stool for α_1 antitrypsin.

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APPENDIX 1

Vitamin A deficiency and WHO classification.

Xerophthalmia:

Xerophthalmia refers to the spectrum of eye signs that are specific to vitamin A deficiency. The prevalence of xerophthalmia is generally used to estimate the prevalence of vitamin A deficiency in a community (2). Xerosis can progress from mild symptoms through to blindness if the vitamin A deficiency is untreated. For urban slum children in Bangladesh the binocular blindness rate due to vitamin A deficiency is estimated to be 20-35/10,000 children (3).

Nightblindness is often the first symptom of vitamin A deficiency and a history of nightblindness is a very good indicator of vitamin A deficiency in some population. The first clinical sign is usually dryness of the temporal quadrant of the eye. Bitot spots, which are patches of the keratinized patches and bacteria next appear on the quadrants of the conjunctiva. The cornea may next become involved with the keratinized patches becoming ulcerated and ultimately leading to blindness.

The World Health Organization has produced a set of criteria for determining if vitamin A is a significant public health problem in a given country.

Classification code	Clinical description	Prevalence level among pre-school children indicating significant public health problem
XN	Nightblindness	1%
XIA	Conjunctival xerosis	.5%
XIB	Bitot's spots	.5%
X2	Corneal xerosis	.5%
X3A	Corneal ulceration involving <1/3 of the corneal surface	0.01%
X3B	Corneal ulceration involving > 1/3 of the corneal surface	0.07%
XS	Corneal scar	0.05%
XF	Xerophthalmic fundus	--
Biochemical	Plasma vit A <10 mg/dl	5%

BUDGET

Protocol: Vitamin A supplementation in the treatment of Shigellosis in children.

PERSONNEL COST — Figures are in US $

Name	% effort	LV/STEP	1992	1993	1994	Total
Dr. M. Shahadat Hossain	20%	NOB/10	2328	2677	2947	7952
Medical Officer	20%	NOA/1	1128	1298	1430	3856
Dr. D. Mahalanabis	5%	-	-	-	-	-
Dr. D. Habte	5%	-	-	-	-	-
Res Fellow(doctor)x 2	100%	Spl Gr	2664	2664	2664	7992
Nurse Fellow x 2	100%	Spl Gr	2016	2016	2016	6048
Health Asstt Fellowx2	100%	Spl Gr	2016	2016	2016	6048
Volunteer x 8	100%	Spl Gr	3360	3360	3360	10080
Sub-total:			13512	14031	14433	41976

SUPPLIES AND MATERIALS

Medicine	1500	1000	500	3000
Hospital supplies	2000	2000	1000	5000
Office supplies	1000	500	500	2000
Nonstock supplies	1500	1000	1000	3500
Sub-total:	6000	4500	3000	13500

OTHERS	500	300	200	1000
Sub-total:	500	300	200	1000

INTERDEPARTMENTAL SERVICES

Transport	1000	500	500	2000
Xerox	1000	500	500	2000
Data analysis	1000	1000	3500	5500
Pt. study/hospitalization	30000	20000	10000	60000
Pathological test	1650	1000	650	3300
Microbiological test	3760	2256	1504	7520
Biochemistry test	17098	10258	6839	34195
X-ray	60	50	40	150
Telex	30	50	20	100
Sub-total:	55598	35614	23553	114765

BUDGET SUMMARY

Items	1992	1993	1994	Total
Personnel cost	13512	14031	14433	41976
Supplies & Materials	6000	4500	3000	13500
Others	200	500	300	1000
Interdepartmental services	55598	35614	23553	114765
Total operating cost:	75310	54645	41286	171241
Capital expenditure	300	200	100	600
Total direct cost :	75610	54845	41386	171841
31% overhead	23439	17002	12830	53271
TOTAL PROJECT COST:	99049	71847	54216	225112

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Phone : 600171-78
Telex : 675612 ICDD BJ
Fax : 880-2-883116
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G.P.O Box 128 Dhaka-1000
Bangladesh.

CONSENT FORM

(TO BE READ INFRONT OF THE LEGAL GUARDIAN)

Dysentery is one of main cause of child mortality in Bangladesh.Recently it is established in different studies that the child mortality rate are several times higher among those who are suffering from vitamin A defeciency. ICDDRB has started a research protocol to see the efficacy of vitamin A in dysenteric illness.In this research protocol children between the age of 1-7 years will receive 200,000 iu of vitamin A in addition to their normal treatment.Investigators will observe the influence of vitamin A over dysenteric illness.We hope that the result of this study will help to treat the children suffering from dysentery and in reducing the incidence of dysentery in future as well.As a reult many children will be benefitted. If you allow your child to participate in this study then you will have to stay in this hospital at least for 5(five) days along with your child. For the purpose of the study we will draw 5ml. of venous blood twice from your child and everyday stool will be collected from the rectum by means of a stick with a cotton at its tip(swab stick). This will not cause any harm to your child.

If you do not allow your child to participate in this study even then your child will receive the best possible treatment in this hospital.

Even after participation you will be allowed to withdraw your child from the research at any time and this will not hamper your child to receive treatment in this hospital.

If you agree then please sign below or give your left thumb impression.

Thanks for your cooperation.

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(Investigator's signature) (Guardian's signature or L.T.I.)

Signature of the witness

1._____ 2._____

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আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
রক্ত আমাশয়ে ভিটামিন 'এ'র কার্যকারিতা গবেষণা প্রকল্প

সম্মতি পত্র

রক্ত আমাশা বাংলাদেশে শিশুদের মৃত্যুর একটি প্রধান কারণ। সাম্প্রতিক বিভিন্ন গবেষণায় দেখা গেছে যে, যে সকল শিশুদের শরীরে ভিটামিন 'এ'র অভাব রয়েছে তাদের মৃত্যুর হার কয়েকগুন বেশী। ভিটামিন 'এ' আমাশা রোগ কতটা কমাতে পারে তা দেখার জন্য আইসিডিডিআর,বি একটি প্রকল্প হাতে নিয়েছে। এতে ১ হতে ৭ বছরের আমাশায় আক্রান্ত শিশুদের হাসপাতালে ভর্তিকালীন সময় প্রচলিত চিকিৎসার সাথে সাথে 'ভিটামিন এ' খাওয়ানো হবে এবং আমাশার উপর এর প্রভাব পর্যবেক্ষণ করা হবে। আমরা আশাকরি এই গবেষণার ফলাফল ভবিষ্যৎ শিশুদের আমাশা কমাতে ও চিকিৎসায় সাহায্য করবে এবং বহু শিশুর উপকার হবে।

আপনি যদি আপনার শিশুকে এই গবেষণায় অংশ গ্রহণ করাতে চান তাহলে আপনাকে এই হাসপাতালে শিশুসহ কমপক্ষে পাঁচদিন থাকতে হবে। গবেষণার প্রয়োজনে আপনার শিশুর নিকট হতে ৫ মিলি করে ২ বার রক্ত সংগ্রহ করা হবে এবং প্রতিদিন আপনার শিশুর মলদ্বার হতে মাথায় তুলা লাগানো কাঠির দ্বারা পরীক্ষার জন্য মল সংগ্রহ করা হবে। এতে আপনার শিশুর কোন প্রকার ক্ষতি হবে না।

আপনি এই গবেষণায় অংশ গ্রহণ করতে না চাইলেও আপনার শিশু এই হাসপাতালের সুচিকিৎসা হতে বঞ্চিত হবে না।

গবেষণায় অংশগ্রহণ করার পরও যে কোন সময়ে আপনি আপনার শিশুর নাম এ গবেষণা হতে প্রত্যাহার করে নিতে পারবেন।

আপনি যদি স্বেচ্ছায় এ গবেষণায় অংশ গ্রহণে রাজী থাকেন তাহলে অনুগ্রহ করে নিম্নে স্বাক্ষর করুন বা বাম হাতের বৃদ্ধাংগুলির ছাপ দিন।

আপনার সহযোগিতার জন্য ধন্যবাদ।

গবেষকের স্বাক্ষর

অভিভাবকের স্বাক্ষর/টিপসহি

তারিখ

তারিখ

স্বাক্ষীর স্বাক্ষর

১। ---------- ২। ----------

Title: Vitamin A Supplementation in the treatment of Shigellosis in children

Summary of Referee's Opinions: **Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.**

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓	(but see Commentary)	
Suitability of Methodolbgy	✓	(but see Commentary)	
Feasibility within time period	✓		
Appropriateness of budget			✓ high cost
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☐
- on level of financial support ☑

I do not support the application ☐

Name of Referee: SHEILA M. GORE Signature: S M Gore

Position: Senior Statistician Date: 30. SEPTEMBER 1991

Institution: MRC Biostatistics Unit, CAMBRIDGE

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(1)

Protocol : Vitamin A Supplementation in the treatment of Shigellosis in children

Commentary : Excellent rationale ; exciting project but excessively costly.

Design : Somewhat misleading to describe design as cross-over because no evaluation of the children takes place after day 5, when placebo-treated infants receive vitamin A for ethical reasons but in such a way as not to unmask the randomized allocation. Rephrase text to emphasise that vitamin A dosing on discharge of children randomized to placebo is for ethical reasons but done in such a way as ~~the~~ maintain double-blinding.

Double-blinding : P17 shows that serum vitamin A will be measured on day 1 (admission and at 5 hours post-treatment). Will all tests be performed together at the end of the trial? If not, detailed description is needed of the precautions taken so that lab. results do not unmask/identify the vitamin A treated children within randomization blocks!

Sample size Data from recent clinical trial adduced to work out trial target and study size for cumulative stool frequency but data from this trial are not cited in support of target for apparently primary outcome ~~clinical~~ improvement (how assessed?) on day 3. Did the newer (not 'newer') antibiotic enhance clinical improvement from 50% to 70% by day 3? Note that enhancement from 50% to 62.5% as target requires 250 children per treatment group. 50% to 70% is a 40% improvement (20/50 failures,

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(2)

Protocol: Vitamin A Supplementation in the treatment of Shigellosis in children cont-)

randomization procedure: Provided that serum vitamin A results do not unmask the randomization while the trial continues, block length can be fixed. Otherwise it must be variable. It is not sufficient to have lab. results delayed until after discharge of index child; they must not be available to investigators until all children randomized in the same block have been discharged. eg. Block lengths 6, 8, 4: results not available for first six children until after sixth child is discharged; for children 7 to 14 until after the 14th child is discharged etc. Otherwise assignment for later children in the block may be knowable in advance eg. if 1st block were A A A P P P!

Randomization mechanism: Unclear, will bottles be serially numbered eg. child 001 (1) and 001 (2: i.e. 2nd dose)
002 (1) and 002 (2) etc.

Who will make up master randomization list; and ensure that it is complied with in making up bottles of vitamin A + placebo. Does taste unmask? If child 001 vomits, how does investigator know what was in bottle 001 (1). Or does each bottle contain 2 doses? Detail please.

Outcome variables: A seventh should surely be 'cumulative frequency of vomiting'. Should cumulative frequencies be measured at beginning or end of 3rd day ~~— presumably~~ the end: if so then specify after 9 other periods of observation.

Open drape: a new concept for me. Picture it?!

Consent form: I must learn French.

Costs: Patient, biochemistry, transport, overheads: all high.

Response to reviewer's comments.

1. **Design. Revised according to comment.**

2. **Double blinding. Vitamin A assay will be performed simultaneously and the result will be masked till study is over. Detail procedure is mentioned in the protocol.**

3. **Sample size. New sample size is calculated and incorporated in the protocol. References as regard to new sample size calculation is also mentioned.**

4. **Randomisation. Revised as suggested.**