ment 1.

Date 2/3/1986

ETHICAL REVIEW COMMITTEE, ICDDR,B.
DHAKA - 12

Principal Investigator Dr. A.N. Alam

Trainee Investigator (if any) 38

Application No. 86-010

Supporting Agency (if Non-ICDDR,B)

Title of Study "ENTERIC PROTEIN LOSS IN CHILDHOOD DIARRHOEA"

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 NA 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
   X 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.

(PTO)

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

Principal Investigator

Trainee

86-010
2/3

SECTION 1 - RESEARCH PROTOCOL

1.	TITLE:	ENTERIC PROTEIN LOSS IN CHILDHOOD DIARRHOEA.
2.	PRINCIPAL INVESTIGATOR:	A.N. Alam
	CO-INVESTIGATORS:	M.A. Wahed , S.A. Sarker, Medical Officer (to be named).
	CONSULTANT:	M. Mujibur Rahman
3.	STARTING DATE:	April, 1986
4.	COMPLETION DATE:	September, 1987
5.	TOTAL INCREMENTAL COST:	US $ 34,306.00
6.	SCIENTIFIC PROGRAMME:	This protocol has been approved by the Nutrition Working Group.

Signature of Programme Head

Date: 10/6/85

7. ABSTRACT SUMMARY:

Abnormal transmucosal protein loss had been observed in various gastrointestinal disorders. Diarrhoeal diseases of different aetiologies have such effect which may contribute and lead to malnutrition. Fecal clearance of α_1-antitrypsin (α_1-AT) will be measured to assess the enteric protein loss in children suffering from diarrhoeal diseases with or without malnutrition. Possibility of intestinal permeability changes contributing to such loss will also be explored. Intestinal permeability will be measured from the ratio of lactulose to mannitol recovered in urine of patients receiving feeds containing both markers. Attempts will also be made to determine the nitrogen losses in urine and faeces.

One hundred children of both sexes between 1-12 years of age admitted to ICDDR,B Dhaka hospital with uncomplicated diarrhoea of various aetiologies will be selected for the present study. Children will be followed at three to four weeks after discharge from the hospital.

8. REVIEWS:

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

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

(c) Director: ----------

SECTION II - RESEARCH PLAN

A. BACKGROUND INFORMATION:

Diarrhoeal disease is still one of the major causes of childhood mortality in developing countries[1]. Community studies in Bangladesh have shown that diarrhoea is responsible for about 30% of all deaths amongst young children[2]. At both the rural and urban diarrhoeal treatment centres of ICDDR,B, the most common causes of diarrhoea in children were found to be rotavirus, entero-toxigenic E. coli, C. jejuni, Shigella[3,4]. Enterotoxigenic E. coli was the most common pathogen detected (20%) in the surveillance study at the Dhaka hospital[4] followed by Rotavirus (19%), Campylobacter jejuni (14%), Shigella (12%) and V. cholerae (6%). Trophozoites of E. histolytica and G. lamblia were each identified in 6% of the patients. Rotavirus and E. coli remained the most common pathogens in toddlers (1-4 years) whereas E. coli, Shigella and E. histolytica were more commonly isolated in older children. These highly prevalent illnesses have been found to be the major determinants of growth retardation and subsequent malnutrition[5,6]. The nutritional impact of diarrhoea may function through different mechanisms, such as: (a) reduction of food intake during diarrhoea due to either anorexia or practice of withholding food[7,8] (b) altered absorption of micro and micro-nutrients[9,14], (c) disturbance of normal metabolic and endocrine functions[10], (d) direct nutrient loss[11], and probably also (e) psychologic (anxiety-apathy) phenomenon equivalent to stress. Most of these factors have been looked into the different investigators except the direct nutrient loss.

Various gastrointestinal disorders have been found to be associated with abnormal transmucosal protein loss[11,18]. But such loss in diarrhoeal disease of various aetiologies have received little attention. In one of the recent investigations carried out at ICDDR,B by using random faecal α_1-antitrypsin measurement, it was possible to document that 87% of patients with shigellosis, 63% with infection due to enterotixigenic E. coli, and 42% with rotavirus had significant loss of protein from plasma into the gut lumen[12]. In the same study, 44% of patients in whom no definite pathogen could be isolated were also found to be losing protein in their stool. Cholera was, however, not associated with protein loss[12,13]. However, the duration of protein loss in diarrhoeal diseases of different aetiologies is not known. In the food absorption study carried out by Molla et al[14], 50-60% nitrogen absorption was observed in certain aetiological diarrhoea in acute stage. However, it could not be conclusively determined if the residual unabsorbed amount of nitrogen was related to an endogenous or an exogenous loss.

Diarrhoeal diseases like any other infection have a pronounced catabolic effect on the body mass[10]. The extent and duration of catabolic losses may vary with the severity of illness and in long standing diarrhoea such losses can lead to severe nutritional deficiencies. Excessive gastrointestinal protein loss may pass unnoticed in well nourished children. However, in chronic malnutrition with marked thinning of the intestinal wall, the protein loss may be more severe. In Bangladesh, where there is high prevalence of undernutrition and an increased risk to contract diarrhoeal illnesses, loss of protein in the latter may also contribute to the former. Moreover, no information is available regarding extent and duration of this loss in malnutrition. An attempt should therefore be made to quantitate this loss in terms of magnitude and duration.

The gastrointestinal tract has been shown to play a significant role in the synthesis of serum proteins. When serum proteins are lost into the gastrointestinal tract, they are catabolized rapidly into their constituent aminoacids which are reabsorbed and made available to the body for resynthesis of proteins. Hypoporteinaemia, however, develops when the rate of protein loss and catabolism exceeds the body's protein synthetic capacities.

The gastrointestinal loss of plasma proteins can be measured with a variety of radiolabelled macromolecules[13,15,16]. These methods are expensive, imprecise and cumbersome and are poorly accepted by patients, physician and clinical chemists. Moreover, the patients are exposed to radioactivity and therefore cannot be used routinely as screening tests or to monitor the course of illness. On the other hand, conventional faecal nitrogen balance studies cannot differentiate exogenous sources as faecal nitrogen excretion may remain in the normal range despite significant serum protein leakage into the intra-luminal space[17]. Alpha $_1$-antitrypsin (α_1-AT), a protease inhibitor synthesized by the liver, is readily detectable in the faeces of newborn infants, children and adults[18,19]. It is neigher degraded by digestive enzymes nor reabsorbed by the intestina. It is present in serum at a concentration of 2-5 g/l[18]. Alpha $_1$-AT comprises approximately 4% of the total serum protein content and has a mol. wt. of approximately 50,000 daltons which is similar to that of albumin. Consequently, faecal α_1-AT excretion should paralled enteric loss of albumin. Ninety percent of the serum's ability to inhibit trypsin resides with this glycoprotein.

This accounts for its current terminology. This half-life of normal α_1-AT may be as long as 4-6 days. About 2 mg of α_1-AT are present in 1 ml of serum[19]. Its level increases during infection, cirrhosis of liver of hormonal stimulation and decreases in massive hepatic necrosis of protein losing lesion[19]. Increased faecal elimination of α_1-AT was associated with coelic disease, intestinal lymphangiectasia and chronic inflammatory bowel disease[20]. In this study, we shall use thia as an endogenous marker of plasma protein loss into the gastrointestinal tract[18]. The method is simple, safe, reproducible, non-invasive and can easily be measured by immunodiffusion technique. Several investigators have found this technique to be useful in diagnosing protein losing enteropathy or abnormal enteric protein loss[18,21-23]. As α_1-antitrypsin unlike ordinary protein, does not undergo change during passage through the gut, it may be accepted as an ideal marker for estimating loss of protein from plasma. This noninvasive technique has also been validated recently against standard isotope method[22].

Acute gastroenteritis may be associated with small bowel mucosal damage[30] and such a loss of mucosal integrity in rotavirus infection was found to be associated with altered intestinal permeability[31]. Correlations between mucosal morphological changes and intestinal sugar permeability were observed both in children with acute diarrhoea[32] and malnourished Gambian children with persistent diarrhoea[33].

Intestinal permeability can be measured from the ratio of lactulose and mannitol recovered in the urine of children after a loading dose of both markers[34]. Both these markers resist hydrolysis by intestinal enzymes and are passively absorbed by different routes across the

intestinal wall - the inert disaccharide lactulose by paracellular pathways and mannitol transcellularly via aqueous pores in the cell membrane. Neither marker is metabolised after uptake, and both are excreted wholly and solely in the urine where recovery approaches 100%[32,33]. Urinary excretion is therefore a measure of intestinal absorption. Recently, this simple, reliable, non-invasive test of intestinal permeability has been employed for assessing mucosal integrity instead of invasive perfusion/ infusion studies or small intestinal biopsy[32,33].

Increased severity of diarrhoea is a major problem among children with protein energy malnutrition. Diarrhoea has been found to lower the serum zinc concentration in infants and children[35]. Significantly low levels of serum zinc have been found in Bangladeshi malnourished children, these levels go further down if associated with diarrhoea[36]. Sodium transport in children recovering from protein-energy malnutrition could be improved by addition of zinc[37]. Zinc deficiency has also been reported in patients with chronic inflammatory bowel disease[38] where malnutrition, protein-losing enteropathy and malabsorption with increased zinc loss in the stool may be responsible for depletion[39]. Various ultrastructural changes with decreased levels of mucosal enzymes and villous atrophy have been observed in zinc deficient experimental animals[40]. These changes also accounted for diarrhoea and altered rates of intestinal secretion of water and electrolytes in the animals that had been reversed by dietary zinc supplementation for 48 hours. It is possible that zinc deficiency may influence the duration and severity of diarrhoea also in humans by improving intestinal mucosal permeability. In this study we would like to investigate the effects of zinc supplementation on mucosal permeability changes in children with diarrhoea.

B. SPECIFIC AIMS:

1. To measure the enteric protein loss and determine the duration and magnitude of such loss in diarrhoea of specific etiologies severity of diseases will be graded according to increased level of α_1-AT clearance (normal clearance 20 ml/day).

2. To study the effect of chemotherapy and zinc supplementation on mucosal permeability changes and enteric protein loss in diarrhoea of specific aetiologies.

3. To observe the relationship between faecal nitrogen loss to α_1-antitrypsin loss in the stool.

C. METHODS OF PROCEDURE:

I. Selection of patients.

One hundred children of both sexes between 1-12 years of age and with history of diarrhoea and/or vomiting for 1-3 days will be selected for the present study. Dark-field positive cases (cholera) and those who had received antibiotics prior to hospitalization will be excluded. Children with high fever (above 103°F), pneumonia, otitis media and other systemic manifestations, such as toxic look, meningism, meningitis, abdominal distension high leucocytosis (leukemoid reation with high band), failling HCT, thrombocytopenia and those who are still breast fed will also be excluded. Measurement of faceal $alpha_1$- AT in infants is not recommended as high levels of this glycoprotein have been demonstrated in human colostrum[24,25].

An informed consent will be obtained from the parents or guardians prior to selection. Detailed clinical examination will be performed on admission. An attempt will be made to screen and select the patients of different aetiologies considering the following criteria:

Rotavirus infection:
- Age: 1-3 years
- H/O onset: Initial persistent vomiting followed by watery diarrhoea and dehydration. Low grade fever.
- Stool characteristics:
  - Naked eye character
  - Acidic in reaction
  - Presence of Neutral fat
  - Non specific microscopic finding.
  - Positive 'Rotazyme' test.

Shigella infection: Fever
Abdominal cramp followed by voluminous watery and then bloody mucoid stools with dehydration. Straining during defaecation. Rectal prolapse, if any.

Stool characteristics: Typical naked eye appearance.

Alkaline in reaction.
Stool M/E: RBC >5/hpf, Pus cells >30/hpf.

ETEC infection: Sudden onset of watery diarrhoea, Nausea, vomiting, Abdominal pain ± low grade fever.

Stool: Alkaline stool with few pus cells and no RBC.

Amoebiasis: H/O intermittent diarrhoea,
Abdominal cramping and flatulence,
Foul smelling bloody-mucoid volumious stool.

Stool M/E: (on finger specimen).

RBC . 30/hpf
Pus cell ;10/hpf
E. hist. trophozoites with ingested RBC.

Typhoid diarrhoea: Continued fever for more than 4-5 days with prostration. Watery diarrhoea without mucus and blood. Abdominal tenderness ± distension. Hepatosplenomegaly.
Leucopenia with relative lymphocytosis
Positive Widal test with 'O' titre of 1:80 or more.

Stool: Alkaline stool with few Pus cells.

II. Management of patients:

Detailed clinical examination will be done on admission and patients will be monitored for change in the vital signs, peripheral blood count or any systemic complications.

Diets will be offered ad libitum (dietary schedule attached) at definite intervals and accurate amount will be calculated after subtracting the left over portion using a Toledo scale (weighing upto 0.1 gm). Duplicate samples from each diet will be saved for nitrogen estimation. Anthropometric measurements e.g. weight and height will be taken in each patient (weight recorded daily and height during discharge). The patient will be discharged after clinical cure and parents will be requested to bring back their children after 4 weeks (recovery stage).

Patients with any degree of dehydration will be initially hydrated and maintained following the WHO guidelines. An accurate intake and output chart will be maintained during the study period.

Depending on the sensitivity report, chemotherapeutic agents (e.g. Ampicillin or Co-trimoxazole or nalidixic acid in patients with Shigellosis, metronidazole in amoebiasis and Chloramphenicol in typhoid fever) will be used according to the following dosage schedule:

Ampicillin	: 100 mg/kg body wt. daily in 4 divided doses for 5 days.
Co-trimoxazole	: Trimethoprim 10 mg/kg and Sulphamethoxazole 50 mg/kg body wt. daily in two divided doses.
Nalidixic acid	: 55 mg/kg body wt. in 4 divided doses for 5 days.
Metronidazole	: 50 mg/kg body wt. in 3 divided doses for 7 days.
Chloramphenicol	: 60 mg/kg body wt. daily in 4 divided doses initially and then reduced to 40 mg/kg body wt. if temperature is maintained at 100°F or less for more than 24 hours. Total treatment will be continued for 14 days.

Alternate patients in each aetiological diarrhoea will receive 100 mmol zinc/kg body wt./day in a syrup mix. The other half will receive a placebo syrup base.

III. Sample collection.

Attempts will be made to do the first phase of the study immediately after the initial rehydration. In patients with shigellosis, amoebiasis and typhoid diarrhoea where antibiotics will be required, the stool and urine samples will be collected in four phases the first two collections will be before and with chemotherapy and the latter two in convalescent and recovery stage. In patients with rotavirus and E. coli infection, the samples will be collected in three phases the first phase will be during the acute illness, the second phase during convalescent stage and the third phase will be during recovery stage.

As soon as rehydration is completed, patient will be fed a charcoal tablet and study diet will be offered. This will be termed as '0' hour. Collection of all samples of stool, urine and vomitus (if any) will be continued till the appearance of the second marker, fed at the end of 48 hours (1st sample).

After this period, close observation will be made on passing soft or formed stool by the patient. This will be termed as convalescent stage. At this stage, a third marker will be fed and diet will be offered as before.

Collection of samples at convalescent stage will be started on appearance of the third marker and be continued till the appearance of the fourth marker-fed at 48 hours after the administration of third marker.

The procedure undertaken for collection of sample in the acute and convalescent stages will be repeated in the recovery stage.

All samples (stool, urine and vomitus) will be collected separately and volume recorded. An aliquot of homogenised stool samples will be stored in the deep freeze until assay for faecal nitrogen and α_1-AT concentration. Urine samples will be stored for nitrogen estimation.

Intestinal permeability tests (as described later on) will be carried out twice in the acute and recovery stages.

IV. Other laboratory Test:

1. Stool will be examined for occult blood, ova or parasites, presence of pus cell, RBC, neutral fat on admission, before discharge and during follow-up.

2. Determination of aetiology of diarrhoeas will be carried out[26]. Toxigenicity of E. coli will be tested by infant mouse assay[27]. Rotavirus will be identified by enzymelinked immunoabsorbent assay[28] using the Rotazyme diagnostic kit. (Abbot Laboratories).

3. 2.0 ml of blood will be collected on admission for routine TWBC, DC, HCT, serum zinc & alkaline phosphatase, α_1-T and electrophoresis of pretein. One ml blood will be drawn at convalescent and recovery stage for α_1-AT and electrophoresis of protein and serum zinc and alkaline phosphase estimation.

4. 4 ml venous blood will be drawn for Widal test and culture in suspected typhoid cases.

5. Faecal and urinary nitrogen estimation will be carried out according micro-kjeldahl procedure[29].

6. α_1-Antitrypsin on lyophilized faecal sample and serum will be carried out according to Crossley and Elliott[18] using commercially available immunodiffusion plates containing monospecific antiserum against α_1-AT. Faecal antitrypsin will be expressed as mg/g dry weight and clearance of serum α_1-AT by the G.I. tract will be determined for each 24 hour period using the formula:

$C = \frac{F \times V}{P}$, where F is faecal α_1-AT concentration in mg/g stool
V is volume of stool in g/day
(clearance=ml/day)& P is the α_1-AT concentration[21] in plasma in mg.

7. The test feeds will contain 4 G lacutose and 0.8 G Mannitol to every 100 ml of water. Children will be encouraged to drink 50 ml of the test feed within two hours. During this period, solid feeds will be withheld. Urine samples for subsequent 5 hours will be collected and an aliquoty (5 ml) will be used for lactulose and mannitol estimation by colorimentric method. Ratio of mannitol over lactulose will be calculated. A normal range of ratio of less than 0.55 will be considered to assess the increased permeability[34].

8. Total N_2 intake will be calculated from the diets given during 24 hours.

Plan for Data Analysis

The basic design could be considered a factorial experinent with four factors (zinc + antibiotic, antibiotic alone, zinc alone and none). Statistical significance for each of these factors may be shown by following standard factorial analysis technique.

Mean α_1-AT clearance in different aetiology will be compared with each other. α_1-AT concentration at the recovery stage will act as control for the same patients.

Relationship between (i) α_1-AT clearance and intestinal permeability changes; (ii) faecal nitrogen and α_1-AT concentration and (iii) faecal cells (puscell and RBC) and α_1-AT concentration will be established by regression analysis.

D. SIGNIFICANCE:

Apart from inadequate intake and altered absorption of nutrients, direct protein loss could be an important factor in causing malnutrition in acute diarrhoea. Information regarding (i) the extent and duration of such loss, its relation with intestinal permeability changes and (ii) the effects of therapy in diarrhoea of specific aetiology will help us in evaluating the prognosis and suggesting interventions in such patients.

E. FACILITIES REQUIRED:

Immunodiffusion plates will be required for determination of α_1-AT nitrogen estimation will be carried out in the Biochemistry Department.

Lactulose and mannitol assay in the urine will be done in the Biochemistry laboratory. Necessary reagents are being ordered. As soon as the method is established, normal excretion values of lactulose and mannitol will be determined in apparently healthy ICDDR,B employees, willing to volunteer, before and after the test feeds.

F. COLLABORATIVE ARRANGEMENTS: None

SECTION III - BUDGET

A. Detailed Budget

1. Personnel services.

Name	Position	% effort	Project requirement (US $)
Dr. A.N. Alam	Principal Investigator	20%	3900.00
Mr. M.A. Wahed	Co-Investigator	15%	1800.00
Dr. S.A. Sarker	Co-Investigator	10%	1000.00
Medical Officer	Co-Investigator	20%	2000.00
Dr. M.M. Rahaman	Consultant	-	-
		Sub Total =	8700.00

2. Supplies and Materials.

20 dozen M - partigen plates (α_1-AT)	6000.00
15 vials standard serum	100.00
Plastic bags	300.00
Stationery goods	100.00
Sub Total =	6500.00

3. Equipment: Reader (Ring diffusion measurement) 25.00

4. Laboratory tests.		Project requirement (US $)
Blood tests (TWBC, DC, HCT)	Tk. 60 X 100	194.00
Stool M/E.	Tk. 40 X 200	260.00
Stool culture for all plates	Tk. 141X 200	912.00
ETEC (ST, LT)	Tk. 45 X 100	145.00
Rotavirus assay (Rotazyme kit)	Tk. 76 X 100	246.00
Urine culture & sensitivity	Tk. 152X 20	98.00
Blood culture	Tk. 209X 20	135.00
X-Ray	Tk. 50 X 50	81.00
Serum albumin	$ 2.00X 200	400.00
Serum electrolytes	$ 3.15X 100	315.00
Serum zinc	$ 3.30X 160	528.00
Alkaline phosphatase	$ 2.40X 160	384.00
Lactulose/ Mannitol assay	$ 4.00X 220	880.00
Kjeldahl Nitrogen	$ 8.7 X 900	7830.00
	Sub Total =	12,408.00

5.	Transportation of patients (Tk. 16 X 30 X 60)	931.00
6.	Patient hospitalization (Tk. 500.00 per day per patient)	12,542.00
7.	Rent, communication and utilities	200.00
8.	Computer services	500.00
9.	Contractual services	Nil
10.	Construction	Nil
11.	Xeroxing, mimeography & Medical illustration	200.00
12.	Miscellaneous (e.g. drugs)	1000.00
	Sub Total =	15,373.00

GRAND TOTAL = US $ 43,006.00

TOTAL DIRECT COST = US $ 34,306.00
(excluding personnel)

(US $ = Tk. 30.93)

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ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE:

1. Our study population will include one hundred children between 1-12 years with diarrhoea. Protein loss in diarrhoea may greatly contribute to malnutrition. Determination of the extent and magnitude of such loss will help us in planning intervention strategies.

2. The only invasive procedure that we will do will be blood drawing. The amount of blood that will be drawn during the study will pose no risk to the patients. All patients will receive adequate treatment. However, on detection of any complication during the study period, the patient will be withdrawn from the study, transferred to the general ward for immediate and adequate management.

3. Not applicable.

4. All records of the patients will be kept strictly confidential with the principal investigator. If data is put on computer tapes, study patients will be referred to by number only.

5. Informed consent (signed or thumb impression) from the authorized legal guardian or parent of the subject will be obtained prior to the study. There is no procedure in this study which may unmask the privacy of the study.

6. Interview will be taken from the guardians only related to their medical history. This will require about fifteen minutes.

7. Each individual will receive best possible care available for his/her illness and this will be the major benefit that will accrue to them through the study. The only inconvenience will be a longer stay than usual in the hospital. Society will however, benefit because we will be able to develop intervention strategies for what is a major cause of malnutrition in children with diarrhoea.

8. This study will require the use of hospital records (that we will record ourselves), stool, urine for blood.

Consent Form

Your child is suffering from diarrhoea/dysentery. Due to this disease, huge amount of protein are being lost in faeces in addition to water and electrolytes. In the present treatment, water and electrolytes are being replaced. But the extent of protein loss is not known. Moreover, it is not clearly understood whether intestinal permeability is changed in this disease and zinc supplementation alters the permeability changes.

With your consent, we would like your child to participate in this study. We expect your full cooperation which will help us to know various informations on your ill child. For the purpose of our study, the following tests will be performed:

1. Immediately after admission, we shall collect 2 ml venous blood (for typhoid patient, additional 4 ml blood will be collected for proper diagnosis of typhoid fever).

2. Stool and urine samples will be collected during 1st 48 hours of admission and another 48 hours at convalescent phase. For timed collection, a harmless marker (Tab. ultra carbon) will be fed. Another 1 ml blood will be collected at convalescent phase.

3. 3-4 weeks after discharge, we would like you to bring your child to the hospital for 48 hours stay. Timed stool, urine and one ml blood will then be collected.

4. To test the intestinal permeability, a 50 ml drink containing two harmless sugars (4 gm. Lactulose and 0.8 gm Mannitol) will be served to your child. No solid diet will be allowed for 2 hours after the drink. All urine upto 5 hours will be collected.

5. To estimate the exact amount of protein content, premeasured diet will be served.

The child will be staying in the hospital until complete recovery and during this period, necessary treatment will be given to your child. There shall be no discomfort to your child because of participation in the study. However, the child may feel little pain during blood drawing.

We are hopeful that the result of this study will help us to carrectly evaluate the effects of diarrhoea/dysentery in causing malnutrition and then initiate appropriate measures to avert such complication.

You may withdraw your child from the study at any time and in that case the treatment will not be hampered. Your further queries regarding the study will always be welcome.

Signature of Investigator

Signature or L.T.I. of patient's guardian.

Date: ----------

সম্মতি পত্র

আপনার সন্তান ডায়রিয়া/আমাশয় রোগে ভুগছে। এই অসুখের কারণে দেহ থেকে মলের সংগে লবণ এবং পানি হারাচ্ছে, প্রচুর পরিমাণে আমিষ জাতীয় পদার্থ বের হয়ে যাচ্ছে। বর্তমান চিকিৎসা পদ্ধতি অনুসারে ঘাটতি লবণ এবং পানি পূরণ করা হয়। কিন্তু আমিষ জাতীয় পদার্থের পরিমাণ (মলে) এখন পর্যন্ত সঠিক ভাবে নির্ণয় করা যায়নি। আমাদের এরূপ ঘাটতির জন্য শরীরের ও আন্ত্রে কোন পরিবর্তন হয় কিনা - তাও জানা যায়নি। এ প্রশ্নগুলোর উত্তর পাবার আশায় আমরা এক গবেষণা কার্যক্রম হাতে নিয়েছি।

আপনার সম্মতি থাকলে, আপনার সন্তানকে এ গবেষণার অন্তর্ভুক্ত করা হবে। হাসপাতালে থাকাকালীন রোগীর বিভিন্ন তথ্যাদি জানার জন্য আপনার সহযোগীতার প্রয়োজন হবে। নিয়ম মাফিক পরীক্ষা এবং প্রয়োজনীয় চিকিৎসা করা হবে। গবেষণার খাতিরে নিম্নলিখিত পরীক্ষাগুলো করা হবে।

১) ভর্তির পর ২ মি:লি রক্ত নেওয়া হবে (টাইফয়েডের রোগীর বেলায় অতিরিক্ত ৪ মি:লি: রক্তের প্রয়োজন হবে টাইফয়েড রোগ নির্ণয়ের জন্য)। এ রক্ত রোগীর স্বাভাবিক চিকিৎসা এবং গবেষণা কার্যক্রম উভয়ের জন্য ব্যবহার করা হবে।

২) ২৪ ঘন্টার মধ্যে মল মূত্র সংগ্রহ করা হবে। এইভাবে সংগ্রহ প্রথম ৪৮ ঘন্টা, রোগী কিছুটা সুস্থ হবার পর পুনরায় ৪৮ ঘন্টার জন্য করা হবে। সঠিক সময় এবং সঠিক ভাবে মলমূত্র সংগ্রহের সুবিধার্থে, অতিরিক্ত এক প্রকার কার্বন ট্যাবলেট (ultra carbon) রোগীকে খাওয়ানো হবে। এ সময় পরীক্ষার জন্য ১ মি: লি রক্ত নেওয়া হবে।

৩) আন্ত্রের সম্ভাব্য কোন পরিবর্তন পরিমাপের জন্য অতিরিক্তভাবে শর্করা জাতীয় শরবত (৪ গ্রাম ল্যাকটুলোজ এবং ০.৮ গ্রাম ম্যানিটল) খেতে দেওয়া হবে। ২ ঘন্টা সময়ের মধ্যে এরূপ শরবত ৫০ মি:লি খেতে হবে, এবং এই সময়ের মধ্যে রোগীকে অন্যকোন খাবার খেতে দেওয়া হবে না। খাবার পরের ৫ (পাঁচ) ঘন্টায় রোগীর প্রস্রাব সংগ্রহ করা হবে।

৪) মাঝে মাঝে রোগীর খাবার পরিবেশন করা হবে যাতে, খাদ্যে মোট আমিষের পরিমাণ নির্ণয় করা সম্ভব হয়।

৫) রোগ সম্পূর্ণ নিরাময় না হওয়া পর্যন্ত রোগীকে হাসপাতালে রাখা হবে এবং উক্ত সময় রোগীর প্রয়োজনীয় চিকিৎসা চলতে থাকবে।

৬) রোগী সুস্থ হলে তাকে ছেড়ে দেওয়া হবে। এর ৩ থেকে ৪ সপ্তাহ পরে রোগীকে আবার হাসপাতালে আসতে হবে। এখানেও পূর্বের ন্যায় ৪৮ ঘন্টার মলমূত্র সংগ্রহ করা হবে এবং ১ মি:লি রক্ত পরীক্ষার জন্য নেওয়া হবে।

গবেষণায় অংশগ্রহণের কারণে রোগীর কোনো রকম ক্ষতি হবে না। কেবলমাত্র রক্ত নেবার সময় রোগী সামান্য ব্যথা অনুভব করবে।

এ গবেষণা . ডায়রিয়া ও আমাদের দেশের অপুষ্টিহীনতার সঠিক মূল্যায়ন এবং ভবিষ্যতে চিকিৎসাপ্রদানে সহায়তা করবে।

আপনি যেকোন সময় এ গবেষণা কার্যক্রম থেকে আপনার সম্মতি প্রত্যাহার করতে পারবেন এবং তাতে রোগী স্বাভাবিক চিকিৎসা থেকে বঞ্চিত হবে না। আপনি যদি আপনার সন্তানকে এ গবেষণায় অংশগ্রহণ করতে নাও দেন, তবুও রোগী তার স্বাভাবিক চিকিৎসা পাবে। আপনি জানতে চাইলে আপনার রোগীর ব্যাপারে বিস্তারিত তথ্যাদি আপনাকে জানানো হবে।

আপনি যদি এ গবেষণায় আপনার সন্তানের অংশগ্রহণে সম্মত থাকেন তাহলে নীচে স্বাক্ষর করুন। ধন্যবাদ।

গবেষকের স্বাক্ষর: ....

তারিখ:

অভিভাবকের স্বাক্ষর/টিপসই ---------------

তারিখ:

Date	Rice powder= 25 G Dano milk =200 G Sugar = 35 G Soya oil = 5 G		Dano milk = 31.2 G Sugar = 15 G		Boiled rice = 250 G Chicken minced=30 G Onion = 10 G Oil = 8 G		Dano milk = 31.2 G Sugar = 15 G		Chicken minced=30 G Onion = 10 G Oil = 8 G		Dano milk=31.2 Sugar = 15 G	
	7:00 a.m.		10:00 a.m.		12:00 noon		3:00 p.m.		6:00 p.m.		9:00 p.m.	
	Offered	Consumed	Offered	Consumed	Offered	Consumed	Offered	Consumed	Offered	Consumed	Offered	Consumed