Attachment 1 A-000030 Library Date 5.9.95

ETHICAL REVIEW COMMITTEE, ICDDR,B. 27/9/95

Principal Investigator Dr. I. Kabir
Dr. G. Fuchs

Trainee Investigator (if any) 28

Application No. 95-022 (Revised)

Supporting Agency (if Non-ICDDR,B)

Title of Study Clinical Efficacy of L-Glutamine in Persistent Diarrhoea 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)
   NA 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.

(PTO)

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

Kabir Fuchs

Principal Investigator

RECEIVED 12 NOV 2001

Trainee

SECTION I - RESEARCH PROTOCOL

1. Title: Clinical efficacy of L-Glutamine in persistent diarrhoea in children.

2. Principal investigators : Dr. A.K.M. Iqbal Kabir
: Dr. George Fuchs

Co-investigators : Dr. Asma Khanam
Dr. Rukhsana Haider
Dr. R.N. Mazumder

Consultant : Dr. M.A. Khaled

3. Staring date : As soon as fund is available

4. Completion date : Two years from starting

5. Total Direct Cost : US $ 176,271

6. Possible source of funding : UNICEF/SDC/Thrasher Fund

7. Scientific Division : This protocol has been approved by the Clinical Sciences Division.

Nplaw

Signature of the Division Director (Actg)

Date: 05 Sept. 95

Abstract Summary

Persistent diarrhoea is a major public health problem in younger children in many developing countries. While death due to acute watery diarrhoea can be reduced substantially with oral rehydration therapy, persistent diarrhoea remains a major cause of diarrhoeal mortality ranging from 15-50%. The etiology and pathogenesis of persistent diarrhoea is complex and multifactorial in which nutritional, infective, and allergic factors are capable of perpetuating the cycle of malnutrition and diarrhoea. Small bowel injury may persist for prolonged periods even after the disappearance of the eliciting factors, particularly in malnourished individuals. Therefore, an adequate mucosal regeneration and repair is a prerequisite of complete clinical recovery.

Contemporary management of persistent diarrhoea includes avoidance of lactose and cow's milk protein, promotion of breast feeding, dietary supplementation with vitamin A, zinc, iron, folate, and vitamin B12, and improved oral rehydration solutions. The role of specific amino acid such as glutamine supplementation in the diet has never been studied in persistent diarrhoea. Several studies demonstrate that glutamine plays an important role in the maintenance of intestinal structure, metabolism, and function. Glutamine supplementation of TPN solution has resulted in increased villous height and DNA content. As glutamine is an essential nutrient for enterocytes, it is important to evaluate the role of glutamine supplementation in the clinical recovery and mucosal repair in children with persistent diarrhoea as we currently propose.

Ninety children with PD (diarrhoea > 14 days) will be given either a rice-based diet containing L-Glutamine supplementation or a rice-based diet with egg albumin for 7 days. Children will be kept in the metabolic study ward, and intake and output will be measured 8-hourly. Lactulose-mannitol ratio will be determined from a 24-h urine collection to determine the effect of L-glutamine on mucosal permeability. The permeability test will be performed before the supplementation and after clinical recovery that is expected 7-days after the dietary therapy. Success of diet supplemented with glutamine will be determined by the passage of soft stool for consecutive 24 h and the proportion of success, difference in the weight from admission and recovery will be compared between the groups. If glutamine supplemented diet shows earlier clinical and nutritional recovery than standard treatment of persistent diarrhoea cases, it is likely to have a substantial impact on child survival by reducing childhood mortality with persistent diarrhoea.

SECTION II - RESEARCH PLAN

Hypothesis

Dietary supplementation of L-glutamine will improve gut permeability by early regeneration and repair of intestinal epithelium in persistent diarrhoea of children and will enhance clinical recovery.

1. **Objective:**

To evaluate the clinical efficacy of L-glutamine, an amino acid which acts as the fuel for enterocytes in children with persistent diarrhoea syndrome.

2. **Background:**

2.1 Persistent diarrhoea: Global magnitude of the problem

Diarrhoeal diseases have long been recognized as leading cause of childhood morbidity and mortality in developing countries (1-3). An estimated 3-5 billion diarrhoeal episodes and 5-10 million diarrhoea-related deaths occur annually among 3 billion people those live in Africa, Asia and Latin America (1). Although the majority of episodes of diarrhoea resolve within 1-2 weeks, a proportion of illnesses persist for a longer period (4). and between 3 and 19% of childhood diarrhoea are prolonged for more than 14 days (4-8).

2.2 Risk factors:

A number of studies examined risk factors and predictors of persistent diarrhoea of which age, nutritional and immunological status have been found to be important. Associated conditions such as dysentery, lower respiratory tract infection, vitamin A deficiency, and use of antibiotic before coming to the hospital have been identified as less important risk factors (17).

Age: Although the effect of age on diarrhoeal duration is not clear, the overall incidence of persistent diarrhoea is usually greater during the first year of life. A study from India showed the incidence of PD was highest in the age group 1-11 months with 31 episodes per 100 child year compared with 9 per 100 child year for 12-23 month age group (7). Similar results are obtained from Peru, Brazil and Guatemala (5,6,9). Age-related factors include maternal antibody for the for 2-3 months of life, weaning age,, child caring practices, crowding etc.

Nutritional status: Diarrhoea and malnutrition are common in developing countries, and either of these conditions put the child at higher risk of dyeing. A number of studies have shown that malnourished children have longer duration of diarrhoea than their better

nourished counterparts (5, 11, 12). Until recently, the role of nutritional status on the incidence of PD was less clear. A case-control study in northern India showed that weight-for-age below 70th percentile was associated with persistent diarrhoea. Another study in northern Brazil showed a significant graded association between poor nutrition as measured by length-for-age or weight-for-age and diarrheal incidence (10). In this study, the average duration of diarrhoea was also significantly longer during the 2-month periods which were preceded by the worst nutritional status. Overall, the increased incidence and increased duration of diarrhoea combined predispose the malnourished children to nearly twice the total number of days of diarrhoea when compared with better nourished children (9, 10).

Immunological status: In a prospective study in Bangladesh it has been shown that in children older than 36 months anergy was associated with 58% increased attack rate and 83% increased duration of diarrhoeal diseases, but not with the respiratory infections. Ninety-three percent of diarrhoeal illnesses were lasting at least 14 days were among anergic children (15). Another study in Lima, Peru showed that delayed cutaneous hypersensitivity responses to seven antigens was inversely related to the incidence, but not with duration of diarrhoea (16). However, in a cohort of 60 children in Brazil showed that diarrhoeal episodes were more in anergic children compared to nonanergic children (M. Castro et al).

Other risk factors: A retrospective analysis of 4,155 children under 5 years showed that 10 percent of these children had persistent diarrhoea. a comparison with children with acute diarrhoea identified 11 factors to be correlated with persistent diarrhoea. More strongly associated factors were presence of blood and mucus in stool, lower respiratory tract infection, malnutrition, vitamin A deficiency, and use of antibiotics before coming to the hospital (17).

Etiology and pathophysiology:

The etiology and pathogenesis of persistent diarrhoea is usually multifactorial, in which nutritional, infective, allergic factors could be perpetuating the cycle of malnutrition and diarrhoea. The leading potential pathogens seen with persistent diarrhoea in some areas are enteroaggregative *E. coli* , identifiable by the characteristic aggregative pattern of adherence of bacteria in HEp-2 cell assay (18-20), *Cryptosporidium* (21). In addition several other pathogens and indeed, multiple or sequential viral, bacterial, parasites, and fungi have been associated with persistent diarrhoea.

The exact mechanism by which enteroaggregative *E. coli* causes persistent diarrhoea is not clear. One study in Brazil by Lima et al. demonstrated 53% of isolated *E. coli* exhibited aggregative adherence to HEp-2 cell, 37% had diffusely adherent *E. coli*, and 16% had locally adherent *E. coli*. Two studies have found an association of bloody diarrhoea and stool isolation of aggregative *E. coli* (22, 23). Of 78 Mexican children from whom aggregative *E. coli* were isolated 32% had blood in their stool (23). Bhan

et al. (19) also found grossly bloody diarrhoea in 12% children with aggregative *E. coli*.

Certain aggregative strains may produce a novel plasmid-associated heat-stable enterotoxin that, with concentrated culture filtrates, elicits short circuit current in Ussing chamber. Alternatively, colonization alone, without morphological alteration of the mucosa, may impair disaccharidase activity, water and electrolyte absorption, and cause diarrhoea, possibly via release of IL-6 (24). Finally, it has been suggested that damage resulting from malnutrition may increase intestinal permeability and allow penetration of epithelium by antigenic materials, which may initiate a local immune response resulting in chronic enteropathy and persistent diarrhoea (R).

Altered Intestinal Permeability in PD:

Intestinal permeability using a dual sugar probe has been used for several years in the investigation of coeliac disease and other small intestinal diseases (26). This technique is based on the fact that under certain conditions, the uptake of probe molecules across the small intestinal mucosa is reflected by their excretion in urine. Because of its small molecular size, mannitol, a monosaccharide, is absorbed transcellularly into the enterocyte. The amount absorbed and ultimately excreted in urine can therefore, be considered to reflect the mucosal integrity, e.g. mannitol absorption would be reduced. On the other hand, Lactulose, being a large molecule, would not be able to penetrate the epithelial cell. But during the small intestinal disease, when mucosal barrier is thought to be disrupted, uptake of Lactulose is increased through the damaged intestinal mucosa, and will excrete in the urine.

Intestinal permeability in Gambian children with persistent diarrhoea as measured by the Lactulose/mannitol excretion ratio was higher (2.85) than those in healthy children (27). Ford et al (25) also found that children with chronic diarrhoea and acute gastroenteritis had an increased permeability. They also found an association of abnormal intestinal permeability with diarrhoeal diseases and with mucosal damage. However, mucosal damage does not always correlate with disaccharidase activity in infants with persistent diarrhoea. Roy et al. (JPGN, 1992) used Lactulose /mannitol permeability test in persistent diarrhea in Bangladeshi infants. They have found increased excretion of Lactulose and reduced excretion of mannitol in both acute and persistent diarrhoea. The Lactulose/mannitol ratio (L/M) was higher in subjects with mucosal invasive pathogens. Two-week zinc supplementation significantly reduced Lactulose excretion in acute and persistent diarrhoea, whereas the change in mannitol excretion and L/M was similar between study groups in both studies.

Intestinal Dysfunction due to Oxidative Stress:

Severe malnutrition is often associated with diarrhoea and malabsorption in children of developing countries. The higher prevalence of diarrhoeal diseases during malnutrition has been explained by impaired immunity and by alteration of gastrointestinal metabolic

activities. Reduction in epithelial cell turnover and enzyme activities has been observed in protein-energy malnutrition and may lead to altered transport of glucose (28) and protein (29). It has been postulated that oxidative stress may be an important cause of intestinal dysfunction in malnutrition. Impaired intestinal permeability and fluid and electrolyte loss are found to be associated with gastrointestinal diseases in which reactive oxygen species (ROS) are thought to play a key role in intestinal ischemia, inflammatory bowel disease, and necrotizing enterocolitis (30). Moreover, recent data on experimental and clinical studies suggest that malnutrition is associated with free radical damage (31). However, little is known about the antioxidant status of gastro-intestine in diarrhoea and malnutrition. It is also known that children with persistent diarrhoea are more likely to be deficient in micronutrients known to have antioxidants properties. Therefore, it is important to evaluate the role of oxidative stress in the pathogenesis of persistent diarrhoea, and thus supplementation of antioxidants (micronutrients, proteins) might be useful in enhancement of clinical recovery.

Management of Persistent Diarrhoea:

Dietary management: Management of persistent diarrhoea remains problematic due to the fact that, exact pathophysiology of PD is poorly understood. Few studies have focused specifically on the clinical and nutritional effects of the dietary management of PD using weaning mixtures prepared from locally available foods. Recent reports from studies in developing countries, using common local vegetable diets to treat acute diarrhoea, show promise for the improved nutritional management of persistent diarrhoea (32, 33, 34, 35).

Micronutrients, such as zinc, vitamin A, glutamine, vitamin B_{12}, folate, iron and possibly others, are involved in intestinal repair, and function as well as varieties of immunological responses. supplementary diets with these micronutrients should probably be given during persistent diarrhoea. Usha et al. (JPGN 1991) showed a clear association of vitamin A with persistent diarrhoea in Indian children.

Recent study from Bangladesh showed that malnourished children with persistent diarrhoea who were given zinc acetate for 2 weeks, had reduction of stool volume accompanied by a shortened duration of diarrhoea. A 2-month follow-up of those children received zinc supplementation also showed significantly fewer episodes of diarrhoea compared with those receiving a placebo. Sachdev in India noted a similar trend with zinc supplementation (36).

Further studies are required to understand the pathogenesis of persistent diarrhoea and its interaction with malnutrition and micronutrient absorption and requirements. Meanwhile the approach to management of persistent diarrhoea must focus on restoring a positive nutrient balance as well as on potential microbial agents in order to restore impaired intestinal mucosal integrity and function. Therefore, it is indeed, pertinent to evaluate the role of L- glutamine (a major mucosal nutrient of small intestine) in clinical outcome of persistent diarrhoea in children.

Glutamine as a gut-specific Nutrient:

Glutamine has been classified as a nonessential amino acid that can be synthesized in the body by a wide variety of tissues. Glutamine has several unique properties that suggest an important role in health and disease. It acts as a precursor in the synthesis of nucleotides and regulator of protein synthesis. It is also avidly consumed by rapidly dividing cells such as enterocytes, lymphocytes and fibroblasts. Several studies demonstrate that glutamine plays an important role in the maintenance of intestinal metabolism, structure, and function (38,39,40). Hwang et al demonstrated that glutamine supplementation of TPN solution caused an increase in jejunal mucosal weight and DNA content. Jacob et al found that a combination of glutamine supplementation and epidermal growth factor showed a synergistic effect in increasing the gut epithelial thickness (42). Similarly, other studies also demonstrated that Glutamine supplemented TPN solutions increased villus heights and nitrogen content. Salloum et al. demonstrated the ability of glutamine-supplemented elemental diets to stimulate intestinal mucosal growth following starvation (44). Fox *et al.* have shown that glutamine-supplementation elemental and enteral diets significantly reduced the severity of methotrexate-induced enterocolitis, as reflected by improved morphometric parameters in the jejunum and colon (37).

Absorption: Most of the glutamine uptake in the gastrointestinal tract occurs in the epithelial cells that line the villi of small bowel. Absorption mostly takes place in the jejunum than ileum. The enterocytes have the highest glutaminase activity of all tissue.

Metabolism: Functionally, glutamine metabolism by the small intestine provides a major energy source for the gut and processes nitrogen and carbon from other tissues into precursors for hepatic uraegenesis and gluconeogenesis. Approximately 64% of glutamine carbon is oxidized to carbon dioxide and other end products lactate, and citrate. Glutamine nitrogen appears in ammonia, alanine, citrulline, and proline. The gut can metabolize glutamine, disposes the generated ammonia via portal vein, and simultaneously oxidizes carbon skeleton to generate energy.

Toxicity: There are concerns about the generation of glutamic acid from glutamine, because of potential toxicity related to the role of glutamine acid as a neurotransmitter. However, the generation of toxic levels of glutamic acid as a result of glutamine infusion seems unlikely. Oral load of more than 50 g/day have been tolerated over long period of time without manifestation of glutamic acid toxicity (45).

Effect of Glutamine supplementation on Mucosal Permeability:

Studies have shown impaired intestinal mucosal integrity in association with persistent diarrhoea (26,27). It is speculated that some of the complications of PD e.g. bacterial and endotoxin transmigration, sepsis and systemic infections may be explained by impaired intestinal permeability. A few studies have shown that glutamine-supplemented diet

reduced endotoxin transmigration and luminal bacterial translocation (37,43). Mucosal permeability to enteric bacterial translocation was also significantly reduced and survival significantly increased. They postulated that by decreasing mucosal damage after methotrexate administration, glutamine supplementation helped maintain the intestinal mucosal barrier and thus reducing systemic mucosal bacteremia, improved survival.

Although L-glutamine has been used as a substrate for oral rehydration solution to promote water and sodium absorption, dietary supplementation with glutamine in persistent diarrhoea syndrome has never been studied. It is, therefore, logical to study whether glutamine supplementation improves the clinical outcome and prevent systemic infections by enhancing repair of gut epithelium.

Dose of Glutamine:

It is evident that glutamine is metabolized at a high rate by the small intestine. The actual rate of intestinal glutamine consumption increases as more glutamine is available in the circulation or the lumen. Enteral feeding are generally preferred method of providing calories and protein to patients who require nutritional support. Total parenteral nutrition results in villus atrophy, a phenomenon which generally reversible when oral feedings are resumed. This observation may provide a biochemical basis, in part, for the use of enteral, as opposed to parenteral feedings whenever possible. There is a concern that glutamine is unstable at room temperature, and generate ammonia and pyroglutamic acid. For this reason glutamic acid has also been excluded from the standard amino acid preparations. In fact, these concerns do not appear to be justified. Even at 37 °C, less than 5% of glutamine degrades in 24-hour. If parenteral nutrition solution are kept at 4 °C until use then significant degradation of glutamine activity is less likely.

What should be the dose of glutamine for the repair of damaged mucosa can not be ascertained due to lack of such study in PDS. However, in different studies doses of 2-4% i.e. 20-40 g/L have been used for TPN. In enteral feeding a dose of 15 g/L L-glutamine has been used with apparent success (37). But it is assumed that to get a significant improvement in mucosal permeability 20 g/L may safely be given, because visceral glutamine requirement may be greater during critical illness, when glutamine metabolism by small intestine is known to be increased. Moreover, generation of toxic levels of glutamic acid seems quite unlikely, since oral glutamine loads of more than 50 g/day have been well tolerated over long periods without manifestation of glutamic acid toxicity (46).

Specific aims:

1. To evaluate whether supplementation of glutamine improve the clinical outcome of children with persistent diarrhoea.

2. To determine whether glutamine has any effect in the repair of damaged epithelium as assessed by dual-sugar permeability test.

3. To determine the oxidative stress in the gastrointestinal tract in children with persistent diarrhoea and malnutrition.

B. SIGNIFICANCE AND POLICY IMPLICATION

During past few years tremendous advances have been made in understanding the etiology, pathophysiology and management of acute watery diarrhoea. Moreover, with the advent of oral rehydration solution the management of acute watery diarrhea has become much simplified and saved millions of children. However, persistent diarrhoea remains a major cause of morbidity and mortality in children and put a heavy diarrhoea burden in developing countries. While the etiology of PD remain uncertain and pathophysiology is multifactorial, further studies are needed to identify the etiology, pathogenesis and its interaction with malnutrition and micronutrients. Meanwhile, approach to the management of PD must focus on restoring a positive nutrition balance to replace the protein, energy and micronutrient deficits.

It is more evident now that the composition of diet and the route of delivery play important roles in maintaining gut structure and function. Recent studies have indicated that gut-specific nutrients may play an important role to improve the intestinal permeability and thus enhance the clinical recovery. Besides, protein and energy amino acids and micronutrients such as zinc, vitamin A, glutamine, and epidermal growth factors may be important for healing of intestinal mucosa.

Several studies have demonstrated that glutamine plays a major role in the maintenance of intestinal metabolism, structure, and function. As it has been shown that intestinal mucosal permeability occurs in PD syndrome and glutamine supplementation might help to regenerate the gut mucosa and thereby enhance clinical recovery and positive nutritional balance. An effective control of PD and improve case management will likely to have a substantial impact on child survival in many developing countries.

Ethical Implication: There is no ethical concern for this study. Patients are expected to be benefited with the dietary intervention. For assays of serum protein and other biochemical parameters 5 ml of blood (maximum) will be required before and after study.

METHODS

Patients Selection Criteria:

Age: 6-24 months.
Sex: only male children will be enrolled as strict intake output balance will be required.
Duration of diarrhoea: More than 14 days but less than 45 days.

Exclusion Criteria:

1. Stool volume less than 40 g/kg body weight during the first 24 hr. of admission.
2. Infants exclusively breastfed will not be included in this study.
3. Prsence of associated systemic infections, viz. pneumonia, sepsis and liver diseases.
4. Frank blood and mucus in the stool.
5. Patients with specific infection such as *Giardia lamblia*, *E. histolytica*, *V. choleare*, and *Shigella* sp.

Baseline examination: A baseline clinical history, physical examination will be done. Before enrolling into the study each patient will be observed for 8 hours. During this period the patients will be offered the standard hospital diet for persistent diarrhoea and will be rehydrated with WHO ORS. During this period patient's stool output will be measured and frequency will be noted. If the patient fails to pass 3 diarrhoeal stools or stool volume less than 40 ml/kg, that patient will not be included enrolled into the study.

Study Design: The study will be double-blind, controlled clinical trial.

Randomization: A randomization list will be prepared by using random number table taking block length of 2, 4 and 6. The randomization list will contain a serial number and a code for one of the diets containing either L-glutamine or a placebo. Serially numbered sealed envelopes corresponding to the serial number of patients to be recruited into the study will contain treatment allocation according to the randomization code. The sealed envelope will be opened after the patient is enrolled into the study and patient will be allocated with the assigned dietary regime.

Sample size calculation: Based on studies done at ICDDR,B the recovery rate of 65% with a rice-based diet, we expect that 90% patients receiving glutamine supplemented diet will recover within 7 days. Taking a significance level of 0.05 and power of 80% to detect a difference in this magnitude 40 patients are needed in each group. Assuming a dropout of 10% a total of 90 patient is required.

Formula used

$$n = \frac{p1\,(100-p1) + p2\,(100-p2) * aB}{(p1-p2)^2}$$

Patient Management and Treatment Protocol:

A detailed clinical history will be obtained and a thorough physical examination will be performed including anthropometric measurements such as length, body weight, mid upper arm circumference. Vital signs will be recorded every 8 hours. Routine clinical care will be provided and regular assessment of the patients condition will be monitored and recorded. Degrees of dehydration will be assessed by WHO criteria (R). Patients with severe dehydration will be given intravenous acetate solution (containing Na-133, K-13, Cl-98, and acetate-48 mmol/L of water). Mild to moderately dehydrated patients will given rehydrated with WHO-ORS solution. during baseline observation and entire study period, stool output, vomits, food intake, and requirement of unscheduled IV fluid will be obtained, and stool output rate will be calculated. Breastfeeding will be continued and will be recorded by test weighing methods.

Diets:

Study patients will get either a rice-based diet (control diet) or a glutamine supplemented rice-based diet (test diet) according to randomization. Detail dietary composition is given below;

<u>Test Diet</u>	
Rice powder	60 g
Egg albumin	30 g
Oil (soybean)	30 g
Glucose	20 g
L-glycine	20 g
MgCl	2 g
NaCl	1 g
Calcium lactate	0.5 g
Energy	665 kcal

<u>Control Diet</u>	
Rice powder	60 g
Egg albumin	50 g
Oil (soybean)	30 g
Glucose	20 g
MgCl	2 g
NaCl	1 g
Calcium lactate	0.5 g
Energy	665 kcal

Values are calculated as gram per litre

As these semielemental diets do not contain complementary micronutrient, a multivitamin and minerals formulation will be given to the children in both groups. Diet will be given 100 ml/kg.d in 2-h frequency.

Laboratory Investigations:

1. Blood for CBC, electrolyte, albumin, SGPT and SGOT will also be done to monitor possible toxic effect of glutamine.

2. Stool microscopical examination for ova and parasites.

3. Stool pH and reducing substances, stool osmolality, electrolytes.

4. Stool culture and sensitivity.

5. Urinary analysis.

6. Thiobarbuteric reacting substances (TBARS) for measurement of oxidative stress in PD.

Intestinal Permeability Test: Before the study diet began, a dual sugar permeability test will be performed using Lactulose/mannitol ratio. The intestinal permeability test will be repeated after one week before discharge to evaluate the role of glutamine supplementation in repair of intestinal damage. For younger children lees than 10 kg a freshly prepared solution of 400 mg Lactulose and 100 mg mannitol in 2 ml of water will be used. Children will be fed 2 ml /kg body weight of this fluid with a disposable glass syringe under direct supervision of an health assistant. The syringe will be rinse twice and fluid will be fed to the child. For older children (body weight more than 10 kg) similarly prepared solution with 7.5 ml (5 g) Lactulose and 1 g mannitol will be made up to 20 ml with 1% chloroform water, will fed to the child under direct supervision of health assistant. Fluid intake and breastfeeding will be encouraged. Urine will be collected over the next 5 h in an adhesive backed pediatric urine collecting bag Total urine volume will be recorded and an aliquot of 3-5 ml will be kept at -20 ° C until analysis. Urinary Lactulose and mannitol will be measured by means of an enzymatic assay described previously (26,27). Recovery of urinary sugars will be estimated as percentage of the initial dose and expressed as a ratio of Lactulose and mannitol.

Management of failure cases:

Those patients who fail to respond to study diet will be managed with a comminuted chicken-based diet or manipulation through elimination of different dietary components and maintaining parenteral hydration and nutrient support following the dietary algorithm at ICDDR,B.

Transfer from the study:

Any patient developing complication such as pneumonia, sepsis, convulsion, enterocolitis will be immediately transferred to the Intensive Care Unit of ICDDR,B and excluded from the study. These patients will be managed with appropriate antibiotic and standard hospital management. Data obtained from these patients will also be analyzed.

Patients Follow-up:

The patients will be asked to come for follow-up after 2 weeks. The permeability test will be repeated and a cow's milk-rice powder formulation will be given as test feed. If the child can tolerate the cow's milk formulation they will be advised to continue this diet at home. For those who fail to respond to test diet will be reverted to Rice-suzi (Rice powder, egg albumin and glucose based diet) based diet and will be again asked to come for follow-up after one week for rechallenge with cow's milk.

Outcome variables:

1. Duration of diarrhoea will be determined as the time required till the passage of soft or formed stool for consecutive 24 hours and no liquid stool thereafter. The liquid stool will be determined as the stool can be poured in a container and take- up the shape of the container. The soft stool as can be poured but does not take-up shape of the container. The formed stool can not be poured in container.
2. Success/ recovery of the dietary intervention will be determined as the cessation of diarrhoea by 7 days of start of dietary therapy.
3. Dietary failure will be determined if diarrhoea does not stop by 7 days.
4. Stool output (g/kg/24 h) over the study period.
5. Amount of I.V. required as a proxy indicator of diarrhoeal severity.
6. Changes in body weight.
7. Changes in Lactulose, mannitol ratio.
8. Changes in TBARS values, as a proxy indicator of reduction of oxidative stress.

Statistical analyses:

Means of stool volume, fluid and food intake, intravenous fluid requirement between two groups will be compared by Student's t-test. Proportion of success or failure at the end of dietary supplementation in two groups will be compared by Chi-square test. laboratory and anthropometric data will be compared between two groups using t-test.

FLOW CHART (SEQUENCE OF TASK WITHIN TIME LIMIT)

1. Staff recruitment — one month
2. Training of staff — two weeks.
3. Pretesting — two weeks
4. Patients enrollment and simultaneous data entry — 20 months.
5. Analysis and report writing — two months.

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ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

Persistent diarrhoea is a major cause of morbidity and mortality in children in many developing countries including Bangladesh. The etiology and pathophysiology of persistent diarrhoea is complex and multifactorial. Dietary management has been found to enhance clinical recovery. The role of specific amino acid such as glutamine supplementation in the diet has never been studied. Several studies have demonstrated that glutamine plays an important role in the maintenance of intestinal structure, metabolism and function. Therefore, it is important to evaluate the role of glutamine supplementation in the clinical recovery and mucosal repair in children with persistent diarrhoea as we currently propose. If glutamine supplemented diet shows earlier clinical and nutritional recovery than standard treatment of persistent diarrhoea cases, it is likely to have a substantial impact on child survival by reducing childhood mortality due to persistent diarrhoea.

1. The appropriate subjects for this study are children of 6-24 age. Because these children suffer from persistent diarrhoea and malnutrition.

2. Physical risk to the subjects for this study includes temporary discomfort associated with small amount of blood collection.

3. Physical risk associated with blood collection will be minimized as much as possible. Indeed these are routinely done in sick patients.

4. Confidentiality of the data collected during this study will be maintained by coding the questionnaires and biological specimens with study identification number. All information will be kept under lock and key.

5. Since the study subjects are minor children, a written consent will be obtained from their parents or legal guardians. For illiterate persons the objective of the study and consent form will be read out and thumb print will be obtained in front of a witness.

6. On admission and during study clinical history, physical examination will be done by the Principal Investigator or Study Physician. This is routinely done in patient care. Interview would not be more than 20 minutes.

7. The individual subject will get benefit by receiving clinical care, dietary supplementation to have a early recovery. The general benefit includes, if this study shows early recovery for persistent diarrhoea, the dietary management of persistent diarrhoea will improve and thus reduce childhood mortality and morbidity.

8. The study will use clinical records, blood, urine, and stool.

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, ঢাকা
মহাখালী, ঢাকা

সম্মতি পত্র

সংক্ষিপ্ত শিরোনাম ঃ দীর্ঘমেয়াদী ডায়ারিয়ার চিকিৎসায় গ্লুটামিন

আপনার শিশু দীর্ঘমেয়াদী ডায়রিয়ায় ভূগছে যা অধিকাংশ উন্নয়নশীল দেশ সমূহে শিশুমৃত্যুর ও অসুস্থতার অন্যতম কারন। বিগত গবেষণায় দেখা গেছে যে খাদ্য পরিবর্তনের মাধ্যমে এই ড়ায়রিয়ায় আরোগ্যলাভে সুফল পাওয়া যায়। বিভিন্ন পরীক্ষা এবং মানুষের উপর গবেষণার মাধ্যমে আরো দেখা গেছে যে, আন্ত্রিক ক্ষতিপূরণে গ্লুটামিনের কার্যকারীতা যথেষ্ঠ। আমরা ধারণা করি যে, খাদ্যের সাথে গ্লুটামিনের মিশ্রণের ফলে অন্ত্রের ঝিল্লি পুনরায় পূর্বাবস্থায় ফিরে যাবে, অতএব আমরা গবেষণা করে দেখতে চাই যে খাদ্যে গ্লুটামিনের মিশ্রণ দীর্ঘমেয়াদী ডায়রিয়ায় আরোগ্য লাভ তরান্বিত করে কিনা।

যদি আপনি আপনার শিশুকে এই গবেষণায় অংশ নিতে রাজী থাকেন তাহলে আপনার শিশুকে এই ওয়ার্ডে ভর্তি করা হবে। আপনার শিশুকে সাত দিন পর্যন্ত গ্লুটামিন মিশ্রিত খাদ্য কিংবা গ্লাইসিন মিশ্রিত খাদ্য খেতে দেওয়া হবে। আমরা আপনার শিশুর কাছ থেকে ভর্তির সময় এবং ৭ দিন পরে ১ চা চামচ পরিমাণ (৫ মিঃলিঃ) রক্ত নেব রক্তের আমিষ এবং অন্যান্য জৈবিক পদার্থ পরীক্ষার জন্য।

আপনি যদি আপনার শিশুকে এ গবেষণায় অংশ গ্রহণ করাতে রাজী থাকেন তাহলে এই পত্রে সই করুন। আপনি ইচ্ছা করলে যে কোন সময় আপনার শিশুকে এ গবেষণা থেকে প্রত্যাহার করে নিতে পারবেন সেক্ষেত্রেও আপনার শিশু এ হাসপাতালে প্রচলিত সকল প্রকার চিকিৎসা সুবিধাদি পাবে।

আপনার শিশুর সকল তথ্য গোপন রাখা হবে।

প্রধান গবেষকের স্বাক্ষর	সই/অভিভাবকের বাম হাতের বৃদ্ধাঙ্গুলীর ছাপ
তারিখঃ.....	তারিখঃ .....

CONSENT FORM

Short-title: Glutamine in Persistent Diarrhoea

Your child is suffering from persistent diarrhoea which is a major cause of mortality and morbidity in many developing countries. Previous studies have shown that dietary modification has resulted in better recovery. Glutamine has been shown to improve intestinal repair in different experimental and human trials. We hypothesize that dietary supplementation with glutamine will regenerate intestinal epithelium and thus we want to investigate whether dietary supplementation of glutamine in persistent diarrhoea can enhance clinical recovery.

If you agree to allow your child to participate in this study, your child will be admitted in this study. Your child will be fed either a glutamine-based diet or glycine-based diet for 7 days. We shall draw 5 ml of blood on admission and another 5 ml of blood after 7 days to measure serum proteins and other biochemical markers.

If you agree to participate your child in this study please sign on this form. If at any stage you wish to withdraw your child from the study, you may do so and in that case your child will get standard treatment offered by this hospital. All information of your child will be kept confidential.

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Signature of the PI	Signature/Left Thumb impression of the guardian
Date: _____	Date: _____

PROPOSED BUDGET
[For two years]

Title: L-glutamine in Persistent Diarrhoea in Children

Amount in US Dollar

Line item		% of effort	Total for 2 yr
Personnel services			
Principal Investigator		25	12,000
Co-Investigator (3)		10 x 3	12,500
Medical Officer (1)		100	12,500
Health Assistant (2)		100 x 2	12,000
Dietician		25	4,334
Health Worker (3)		100 x 3	2,300
Data Entry Technician		100	6,100
Secretarial service		10	624
	Sub-total:		62,358
Consultant's travel			5,000
Ticket cost [Chapel Hill-Dhaka & back] + 2 wks per diem at Dhaka, Bangladesh			
International travel (for presentation of study results at Scientific Conference/Seminar)			5,000
Local travel (for patients follow-up)			3,500
Supplies & Materials			
Drugs			4,000
Hosp/Lab supplies			3,000
Stationeries			2,000
	Sub-total:		9,000
Other contractuals			
Fax, e-mail, postage etc.			2,500
Airfreight for transporation of samples (Glutamine, GSH)			500
Priting & publication			500
	Sub-total:		3,500
Inter-departmental services			
Laboratory costs: Stool culture, osmolality, glutamine, GSH, lactulose, mannitol, CBC, Urine			20,000
Land transport (Dhaka), photocopy, library service, illustration, maintenance			5,000
Patient hospitalization cost	@$20 x 90 pts x 9 d		16,200
	Sub-total:		41,200
Capital expenditure			
One pc and one printer with accessories			5,000
TOTAL DIRECT COST			**134,558**
Overhead (31%)			41,713
TOTAL PROJECT COST			**176,271**

5/9/95