Attachment 1. Date 23.9.81.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A. N. Alam Trainee Investigator (if any) 21

Application No. 81-042 Supporting Agency (if Non-ICDDR,B) _____

Title of Study "The role of prostacycline in the development of Haemolytic-Uremic syndrome in acute shigellosis."

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes (No)
   (c) Minors or persons under guardianship (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects (Yes) No
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects (Yes) No
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) (Yes) No
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used Yes No NA
   (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)
   4 ✓ 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.

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

81-042
Rec'd 25/9/81

SECTION I - RESEARCH PROTOCOL

1. Title: The role of prostacycline in the development of Haemolytic-Uremic syndrome in acute shigellosis.

2. Principal Investigator: Dr. A. N. Alam

3. Co-Investigators: Dr. M. R. Islam, Dr. M. M. Rahaman & Medical Officer (to be named).

4. Starting Date: October, 1981

5. Completion Date: September, 1982

6. Total Direct Cost: $ 9000.00

7. Scientific Programme Head:

This protocol has been approved by the Pathogenesis and Therapy Working Group.

* Signature of Scientific Programme Head: W. B.

Date: 22/8/81

This signature implies that the Scientific Programme Head takes responsibility for the planning execution and budget for this particular protocol.

8. Abstract Summary:

The purpose of this study is to determine the role of prostacycline (PGI_2) in the causation of haemolytic-uraemic syndrome (HUS) in children with acute and severe shigellosis. We will test the hypothesis that disturbed haemostasis seen in HUS patients might be related to the dificiency of a plasma

factor which stimulates the activity of vascular prostacycline - the most potent inhibitor of platelet aggregation. This project will begin to study two groups of patients ~~——~~ those with severe shigellosis prone to develop HUS and those with moderately severe uncomplicated shigellosis without HUS who will serve as control. Estimation of 6-keto-$PGF_{2\alpha}$, the sole degradation porduct of PGI, will be done by radioimmunoassay using 1 ml. peripheral blood in both groups of patients. The role of prostacycline in the causation of HUS in acute shigellosis will be assessed by comparing the levels of 6-keto-$PGF_{1\alpha}$ in both HUS and control group of patients.

9. Reviews:

(a) Research Review Committee: _____

(b) Research Review Committee: _____

(c) Director: _____

(d) B.M.R.C.: _____

(e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. Introduction:

1. Objective: The objective of this study will be to determine the pathogenesis of HUS in shigellosis - more specifically the role of prostacycline metabolism in the causation of Haemolytic-Uraemic Syndrome. (HUS)

2. Background: Shigellosis continues to be a major clinical entity and HUS is an extremely important yet unsolved problem in acute shigellosis. HUS is a clinical syndrome characterized by falling haematocrit thrombocytopenia, red cell fragmentation and reticulocytosis, coombs-negative microangiopathic haemolytic anaemia, hypofibrinogenaemia and acute renal failure. First reported by Rahaman et al[1] in 1975, Koster and co-workers[2] reported from this centre the association of endotoxaemia (as defined by the linulus test), intravascular coagulation, circulating immune complexes and postmortem finding of fibrin deposition in glomeenli and renal arteries in many of these patients. Eight of the nine patients had severe grade of colitis and more than half of these patients died. The complication occurred in about 10% of hospitalized children, less than 2 years old, who showed a leukaemoid reaction (TWBC 50,000 per cumm) and had stool cultures positive for Shigella dysenteriae type 1 (shiga bacillus). It occurred most often in the second week of illness when the patients are afebrile and are recovering from an acute

episode of diarrhoea. The data generated from their study supported the hypothesis that severe colitis is associated with circulating endotoxin, which in turn may produce coagulopathy with renal microangiopathy and haemolytic anaemia. However, the exact cause of this serious complication is not yet clear. Recent work by Remuzzi et al[3] suggested that the disturbed haemostasis observed in HUS patients might be related to the deficiency of a plasma factor which stimulates the activity of vascular prostacycline - the most potent inhibitor of platelet aggregation. Prostacycline has been shown to be increased in the bleeding diathesis of uraemia[4] and its defective activity could contribute to the formation of platelet thrombi in the microcirculation - a finding typical of HUS. Determination of prostacycline ideally requires biopsy from peripheral veins[4]. This may be difficult to be obtained from children patients in Bangladesh on ethical grounds. Moreover, PGI_2 has a half-life in vivo at $37^{o}C$ of only 2-3 monutes. However, recently a new method has been described to measure 6-keto-$PGF_{1\alpha}$ - the stable non-enzymatic sole degradation product of PGI_2-in the circulation adapting a radioimmunoassay[5].

B. SPECIFIC AIMS:

(1) Estimation of 6-keto-$PGF_{1\alpha}$, plasma fibrinogen, thrombin time (TT) and fibrinogen-fibrin dedgradation products (FDP) in patients with severe shigellosis with high leucocytosis during the illness and after recovery.

(2) Estimation of presence of endotoxaemia using limulus assay.

C. METHODS OF PROCEDURE:

Selection of patients:

Two groups of patients will be studied:

(1) Group 1 will include patients with severe shigellosis presenting with hypoproteinaemia, hyponatraemia and high leucocytosis (>50,000/cm). These are the high risk patients a higher proportion of whom may eventually develop HUS.

(2) Uncomplicated, moderately severe shigellosis patients without HUS will be selected to act as control group.

Twenty patients in each group, matched for age, sex and duration of diarrhoea prior to admission, will be studied. Patients with severe malnutrition and other complications will be excluded from the study.

Procedure after admission:

(a) A flow sheet (attached with the protocol) will be designed to record the relevant laboratory data.

(b) Plasma level of 6-keto-$PGF_{1\alpha}$ will be determined by radioimmunoassay following the method described by Salmon[5]. 1 ml. of peripheral blood will be drawn from each patient.

(c) Routine finger blood tests e.g. TWBC, DC, HCT, RBC fragmentation, reticulocyte and platelet count will be done.

(d) Electrophoresis of protein, serum electrolytes, urea and creatinine will be done as routine essential clinical investigations. These tests will require 2 ml of venous blood.

(e) Limulus assay for endotoxaemia will be done in patients at the time of admission and again three to seven days later. 1 ml. of venous blood and commercially available limulus lysate (Sigma) will be required for the test.

(f) Measurement of plasma fibrinogen, thrombin time and fibrinogen-fibrin dedgradation products will be done using commercial test kits (Hoechst-Behring). Haptoglobin will be estimated by Single-radial-immunodiffusion (SRID) technique using M-partigen plates (Hoechst-Behring). Total of 2 ml. blood will be required for these estimations.

(g) Urine analysis will also be done in all cases.

(h) Stool for microscopic examination and culture for shigella.

The above mentioned investigations will be done in each patient on admission into hospital, at the height of leucocytosis and subsequently once every week until complete recovery.

Patients will be followup up 2 weeks after complete recovery.

D. SIGNIFICANCE AND RATIONALE:

Estimation of 6-keto-PGF$_{1\alpha}$ should provide us with valuable information about the activity of prostacycline and its possible role in the formation of platelet thrombi in HUS. This will enable us to understand better the pathogenesis of this serious and fatal complication of acute shigellosis.

E. FACILITIES REQUIRED:

1. Laboratory facilities in the clinical pathology, biochemistry and microbiology department will be utilized. Liquid Scintillation counter (Beckman LS 150) available in the biochemistry department will be used for the radioimmunoassy.

2. Patients will be admitted in the study ward.

F. DATA ANALYSIS:

It is anticipated that the following data analysis will be performe

(a) Clinical comparability of the HUS patients and the control group.

(b) Difference in the level of PGI_2 in these two groups. Students "t" test will be applied to see the difference in PGI_2 activity between the HUS patients and the control group.

FLOW SHEET

Case No.: _____

	On admission	Daily	At the height of leucocytosis (>50,000)	1st.wk.	2nd wk.	Follow-up
Blood: TWBC:						
DC						
Hct						
RBC fragmentation						
Reticulocyte ct.						
Platelet ct.						
Electrophoresis of protein						
Sodium						
Potassium						
Chloride						
Bicarbonate						
Urea						
Creatinine						
6-keto-PGF$_{1\alpha}$						
Limulus assay						
Plasma fibrinogen						
Thrombin time						
F.D.Products						
Stool: M/E						
Culture						
Urine analysis						

References:

1. Rahaman MM, Alam AKMJ, Islam MR, Greenough III WB and Lindenbaum J: Shiga Bacillus Dysentery Associated with Marked Leukocytosis and Erythrocyte Fragmentation. The Johns Hopkins Medical Journal 136:65, 1975.

2. Koster F, Levin J, Walker L, Tung KSK, Gilman RH, Rahaman MM, Majid A, Islam S and Williams RC: Haemolytic-Uraemic Syndrome after shigellosis, New Eng J Med 298:927, 1978.

3. Remuzzi G, Marchesi D, Mecca G, Misiani R, Livio M, De Gaetano G and Donati MB: Haemolytic-Uraemic Syndrome: Deficiency of plasma factor(s) regulating prostacycline activity? The Lancet II: 871, 1978.

4. Remuzzi G, Mecca G, Cavenaghi AE, Donati MB and De Gaetano G: Prostacycline-like Activity and Bleeding in Renal Failure. The Lancet II: 1195, 1977.

5. Salmon JA: A radioimmunoassay for 6-keto-prostaglandin $F_{1\alpha}$ Prostaglandins 15:383, 1978.

SECTION III - BUDGET

A. Detailed Budget

1. Personnel:

Name	Position	% Effort	Taka	$ Dollar
Dr. A. N. Alam	Investigator	50%		5385
Dr. M. R. Islam	Co-Investigator	20%		2137
Medical Officer	do	50%		1832
Dr. M.M. Rahaman	do	5%		—
Research Officer(Biochemistry)		30%		1042
Lab. Technicians:				
Biochemistry		30%	12000	
Cl. Pathology		10%	8000	
Microbiology		5%	4000	
Nurse (Study Ward)		30%	10000	
			Tk.34,000	$ 10,396

2. Supplies and Materials:

A.	Limmulus lysate		200
B.	RIA kits		500
C.	Commercial kits for estimation of plasma fibrinogen, thrombin time, FDP & haptoglobin		1000
D.	Lab. tests (electrolytes, urea, creatinine and electrophoresis of protein for each patient)	4800	
E.	TWBC, DC, HCT, plateles & reticulocyte c[illegible]	3000	
F.	Urine analysis	1000	
G.	Stool M/E	800	
F.	Stool culture	3200	
		12,800	1,700

3. Equipment: Nil

4. Hospitalisation cost: 60,000

400 patients days
(10 days x 40) x Tk.150/day

		Taka	$ Dollar
5.	Outpatient care: Nil		
6.	ICDDR,B transport:	1600	
7.	Travel: One round-trip air ticket for bringing a consultant:-		2000
8.	Transportation of materials:		200
9.	Rent, Communication, Utilities: Nil		
10.	Printing and Reproduction:		450
11.	Other contractual services: Nil		
12.	Construction: Nil		
	TOTAL =	Tk. 74,400	$ 4,350
	($ 1 = Taka 16) =	$ 4650	

Total incremental cost = $ 9000

BUDGET SUMMARY

		Tk.	$
1.	Personnel	34,000	10,396
2.	Supplies & Materials	12,800	1,700
3.	Equipment	-	-
4.	Hospitalization cost	60,000	-
5.	Outpatient care	-	-
6.	ICDDR,B Transport	1,600	-
7.	Travel	-	2,000
8.	Transportation of materials	-	200
9.	Rent, Communication, Utilities	-	-
10.	Printing & Reproduction	-	450
11.	Other contractual services	-	-
12.	Construction	-	-
	Total incremental cost	74,400	4,350
		= $ 4,650	

TOTAL = $ 9,000

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

A clinical trial is proposed on 40 children to investigate the role of prostacycline (PGI_2) for the production of haemolytic-Uremic Syndrome (HUS) as a complication of acute shigellosis. We like to test the hypothesis that disturbed haemostasis seen in HUS patients might be related to the dificient activity of vascular prostacycline- the most potent inhibitor of platelet aggregation. We would like to study two groups of children, 20 in each group, matched for age, sex and duration of diarrhoea prior to illness:

(a) those with severe shigellosis likely to develop HUS &

(b) those with moderately severe uncomplicated shigellosis without HUS who will serve as control.

Patients with severe malnutrition and other complications will be excluded from the study.

These patients will be admitted in the study ward and regular clinical evaluation will strictly be done every four hours. 1 ml of blood will be required at the peak of disease to determine the plasma level of 6-keto-$PGF_{1\alpha}$ by radioimmuno assay. Plasma fibrinogen, thrombin time, fibrin-fibrinogen degradation products and haptoglobin will be estimated by using commercial kits. 4 ml of blood will be necessary for these and other essential routine investigations e.g. serum electrophoresis, electrolytes, urea & creatinine. 1 ml of blood will be required on admission, again 3-7 days later for limulus assay for endotoxaemia. Routine finger blood tests like TWBC, DC, HCT, RBC fragmentation, reticulocyte platelet count will be necessary to follow the course of the disease. Stool M/E and culture for shigella will be done on admission.

The above blood examinations will be done on admission, at the peak of leucocytosis and subsequently once weekly until complete recovery in the first group of patients. These patients will be followed up after 2 weeks and all investigations will be repeated.

This study will provide us valuable information about the activity of prostacycline and its possible role in the formation of platelet thrombi in HUS.

In view of the serious nature of the illness which has a high fatality rate, the pathogenic mechanism involved in the development of haemolytic-uremic syndrome could be established from this study so that future therapeutic measures could be developed to save the life of these otherwise fatal patients.

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Prostacycline - HUS study

CONSENT FORM

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out studies to know more about Shigella dysentery. In this study, we aim to know the role of a chemical substance called prostacycline in producing Haemolytic - Uraemic Syndrome, which is a very serious complication seen in Shigella dysentery. We would like your child to join in this study.

If your child participates in this study, the following may be expected:

1. Your child will be given best possible medical care like other patients in this hospital.

2. You will be required to stay in the hospital until complete recovery.

3. During the stay in hospital, we will take 6 ml of venous blood for different laboratory tests on admission. Some of these tests will be repeated 3 - 5 days later and then once weekly until the patient has fully recovered.

4. We will take few drops of blood from the finger of your child on different days for laboratory tests.

5. We will do culture for stool to find out organisms responsible for the dysentery.

6. You will have to return with your child 15 days after discharge, when the above tests will be repeated.

7. If you do not like to include your child in this study, there will be no variation of usual treatment.

8. You will be free to withdraw your child anytime from the study, without any change of the therapy.

If you are willing to participate in this study, please put your signature or thumb impression below.

Signature of Investigator: _____

Signature/Thumb Impression of Guardian: _____

Date: _____

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র

বাংলাদেশ

প্রকাশাইট্রিক - এইচ, ইউ, এস, আই

সম্মতি পত্র

এই গবেষণা কেন্দ্র বিশেলায়িত রক্ত আমাশয় রোগ সম্বন্ধে আরো জানার জন্যে গবেষণা চালিয়ে যাচ্ছে। এই গবেষণায় আমরা বিশেলায়িত রক্ত আমাশয়ের একটি মারাত্মক জটিলতা এইচ, ইউ, এস'র সাথে প্রকাশাইট্রিক নামক একটি রাসায়নিক পদার্থের সম্পর্ক জানার চেষ্টা করছি। আমরা আশা করি আপনার সন্তান এই গবেষণায় অংশগ্রহণ করবে।

এই গবেষণায় অংশগ্রহণ করলে নিম্নলিখিত বিষয়গুলো আশা করতে পারেন:

১) অন্যান্য রোগীর মত আপনার সন্তানও সকল প্রকার চিকিৎসা সুবিধা পাবে।

২) রোগী সম্পূর্ণ সুস্থ না হওয়া পর্যন্ত হাসপাতালে থাকতে হবে।

৩) হাসপাতালে থাকাকালীন অবস্থায় বিভিন্ন পরীক্ষার জন্যে রোগীর শিরা হতে সি, সি, রক্ত নেয়া হবে। এই পরীক্ষা রোগীর ভর্তির সময়, তার ৩-৫ দিন পরে এবং তারপর প্রতি সপ্তাহে একবার করে করা হবে।

৪) বিভিন্ন সময়ে ল্যাবরেটরী পরীক্ষার জন্যে রোগীর আংগুল হতে কয়েক ফোঁটা রক্ত নেয়া হবে।

৫) রক্ত আমাশয়ের সার্বিক কারণ নির্ণয়ের জন্যে রোগীর পায়খানা পরীক্ষা করা হবে।

৬) হাসপাতাল থেকে ছুটি নেয়ার ১৫ দিন পর আবার রোগীকে নিয়ে আসতে হবে। তখন উপরোক্ত পরীক্ষাগুলো আর একবার করা হবে।

৭) এই গবেষণায় অংশগ্রহণ না করলেও আপনার সন্তানের সুচিকিৎসার কোন ব্যতিক্রম হবে না।

(অপর পৃষ্ঠা)