ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 21.3.84
22.3.84

ncipal Investigator Dr. Asma Khanam

Trainee Investigator (if any)

ication No. 84-013

Supporting Agency (if Non-ICDDR,B)

e of Study TYPHOID FEVER: Determinat- of C-AMP and prostaglandin production ng diarrhoea and comparative therapeut tial with Chloramphenicol and riaxone

Project status:
(✓) New Study
(✓) Continuation with change 22
( ) No change (do not fill out rest of form)

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

Source of Population:
(a) Ill subjects — (Yes) No
(b) Non-ill subjects — Yes No
(c) Minors or persons under guardianship — (Yes) No

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)

oes the study involve:
) Use of records, (hospital, medical, death, birth or other) — (Yes) No
) Use of fetal tissue or abortus — Yes No
) Use of organs or body fluids — (Yes) No

subjects clearly informed about:
Nature and purposes of study — (Yes) No
Procedures to be followed including alternatives used — (Yes) No
Physical risks — (Yes) No
Sensitive questions — Yes No
Benefits to be derived — (Yes) No
Right to refuse to participate or to withdraw from study — (Yes) No
Confidential handling of data — (Yes) No
Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will signed consent form be required:
   (a) From subjects — (Yes) No
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — Yes No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ✓ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

na Khanam
ncipal Investigator

22 MAR 1984

SECTION I - RESEARCH PROTOCOL

1. TITLE: TYPHOID FEVER: Determination of c-AMP and prostaglandin production during diarrhoea and comparative therapeutic trial with Chloramphenicol and Ceftriaxone

2. PRINCIPAL INVESTIGATOR: Dr. Asma Khanam

   CO-INVESTIGATORS: Dr. T. Butler, Dr. P. Speelman, Dr. S.K. Nath, Dr. N. Haque, Dr. I. Kabir, Dr. A. Molla,

3. STARTING DATE: 16 April 1984

4. COMPLETION DATE: 15 April 1985

5. TOTAL DIRECT COST: $22852

6. SCIENTIFIC PROGRAMME HEAD: This protocol has been approved by the Pathogenesis and Therapy Working Group.

Signature of Scientific Programme Head: _____

Date: 21 March 84

7. ABSTRACT:

50 cases of typhoid fever aged 6 months to 60 years with diarrhoea will be studied. Selection for the study will be based on history, physical examination, suggestive of typhoid fever and also positive Widal test. Laboratory investigation including blood culture, complete blood count, electrolytes, stool microscopy and culture for all enteropathogens, urine for analysis and culture will be performed on admission. These tests will be repeated during and after therapy is completed. Patients will be followed daily by recording vital signs, stool frequency, consistency and volume every eight hours. Daily physical examination will be performed to monitor the clinical changes and effect of anti-microbial therapy. Besides this drug level in the blood will be measured to record toxicity. Patients will be treated with Chloramphenicol in dose of 60 mg/kg/day till defervescence and then 40 mg/kg/day for 14 days or Ceftriaxone 3 gm. daily for 7 days by randomly assigning the patient to one of the treatment groups. Clinical efficacy will be evaluated by time of defervescent, treatment failure rates, relapses, bacteriological cure and side effects of the drugs used. Patients will be rehydrated with isotonic intravenous solution (Acetate). Intake and output charts will be maintained every 8 hours. Haematocrit, W.B.C., platelet and reticulocyte count will be done before, during and after initiation of therapy. The usual hospital diet will be allowed.

Cyclic AMP and prostaglandin (PGE_2) from ileal fluid aspirate will be measured in 5-10 adult male cases of typhoid fever with diarrhoea at 24 hours of admission (acute) and after 3 weeks (convalescent) to understand the mechanism of diarrhoea.

8. REVIEWS:

a) Research involving human subjects:

b) Research Review Committee:

c) Director:

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

a) To understand the mechanism of diarrhoea in typhoid fever and determine whether this diarrhoea is mediated through c-AMP and prostaglandin.

b) To compare clinical efficacy and safety of ceftriaxone and chloramphenical in the treatment of typhoid fever.

2. Background:

This protocol is a continuation of protocol No. 82-014 based on WHO/UNDP'S recommendation to perform study in 100 cases. In the first 50 cases that were studied associated diarrhoeal pathogens were identified only in 6 cases. 50 additional cases were considered necessary to clarify the rates of associated diarrhoeal pathogen excretion. Besides more work on diarrhoeal mechanisms was also needed.

The clinical features of typhoid fever, pathogenesis, immunology and evidence of diarrhoea in typhoid fever have been described in detail in protocol No. 82-014.

Previous work:

Some of the results of the 50 completed cases studied reveals that typhoid fever is associated with diarrhoea. The mean pre-hospital duration of diarrhoea was 6.8 days, the in-hospital duration of diarrhoea was 4.6 days.

On admission the mean purging rate was 44ml/kg body weight/day. The stool of these patients showed a type of diarrhoea somewhere between invasive and non-invasive type. On admission 32% of patient's stool contained a significant number of faecal leucocytes, 6% had 10 RBC's/hpf in the faecal specimen. The mean quantitative faecal WBC count was 5.160/cumm with 71% poly morphonuclear leucocytes and 29% mononuclear cells. The stool PH was acid (mean 6.1). The mean sodium concentration in the stool was 50.1 meq/l, potassium 47.2 meq/l, chloride 41.5 meq/l and total Co_2 22.9 meq/l.

The frequency of associated pathogen was low. In 53% of the patients the stool culture was positive for S. typhi. Shigella boydei was isolated from 2 specimens. E. coli isolates from 18 patients were tested for enterotoxigenicity of which one was positive for LT, and one for both LT and ST. None were ST positive. No other bacterial co-pathogens were isolated. Trophozoites of E. histolytica was found in the specimen from 2 patients.

S. typhi strain from 20 patients tested for enterotoxigenicity were all found to be negative for LT and ST. Eleven S. typhi strains tested for sereny-test were all negative. Cyclic AMP concentrations in serial stool samples of 6 patients were found to be elevated. In some cases c-AMP concentration of 1500 picomoles/ml was noted. This rise was even higher than the mean levels of 1248 picomoles/ml in cholera patients (17) suggesting a possible role of the adenylate cyclase-cyclic AMP system in pathogenesis of typoid-diarrhoea. Nevertheless the role of prostaglandins in typhoid-diarrhoea should be considered because prostaglandin E_2 (PGE_2) can stimulate adenylate cyclase. (15,16).

These data characterize the cellular and biochemical composition of diarrhoea in typhoid fever. The low frequency of co-pathogens (bacterial and protozoal) in the stool of these patients in association with S. typhi suggest that the infection with S. typhi per se may be responsible for the diarrhoea in these patients. No invasive and enterotoxin activity was demonstrated by the S. typhi strains. Hence the present protocol attempts to study other possible diarrhoegenic mediators e.g. c-AMP and prostaglandin involved in increased intestinal secretion.

The pathogenesis and pathophysiology of salmonella-induced diarrhoea have been studied in rats, rhesus monkeys and rabbits. Rats infected with salmonella typhimurium resulted in new water secretion in the ileum, persistent abnormalities of colonic water, Na or Cl transport did not occur. (1). Despite normal colonic absorptive function, significant alteration of the histologic appearance of the mucosa was observed in this experiment. Rhesus monkeys inoculated with S. typhimurium developed changes of water, Na and Cl movement in jejunum, ileum and colon. Net fluid secretion in the colon was always observed together with a marked colitis that was characterized by micro-absecesses and epithelial disruptions but with only an occasional ulceration (2,3,4). Studies in the ileum of salmonella-infected rabbits indicate that tissue invasion alone was not responsible for the production of net fluid secretion and active ion secretion was also present. These experiments suggest that cyclic AMP and prostaglandin may mediate the observed active secretory process (5,6). Prelininary studies on measure of intraluminal PGE_2 levels in cholera patients by using the fasting intestinal flow rate of PGE_2 as an arbitrary measure of the PGE_2 production rate, found PGE_2 levels and the

intestinal flow rate to be increased in acute cholera. (Unpublished results by Speelman, Rabbani, Bukhave and Madsen 1983).

Several prostaglandins (PGE_2) have been reported to affect fluid and electrolyte movement in the small intestine (7,8,9). Indomethacin, a potent inhibitor of prostaglandin synthesis (10,11) has prevented colonic fluid and electrolyte secretion in salmonella-infected monkeys (2). Indomethacin given parenterally in non-infected monkeys resulted in increased net-water transport in the jejunum, ileum and colon. Only the changes observed in the ileum and colon were statistically significant. But the effect of indomethacin on water transport in salmonella-infected monkeys had more marked changes in ileum and colon after 1 to 2 hours of indomethacin administration in comparison to the non-infected monkeys. (2).

It may be hypothesized thatintestinal infection with invasive salmonella may result in increased local prostaglandin sythesis as a result of mucosal inflammatory reactions (12) and acute inflammatory reactions elsewhere resulting in the synthesis and release of prostaglandin (13,14). Prostaglandins can stimulate intestinal adenylate cyclase activity (15,16) and fluid secretion. Indomethacin abolishes salmonella mediated adenylate cyclase activation and fluid secretion. Unlike the situation in salmonellosis where indomethacin treatment abolished both fluid secretion and activation of adenylate cyclase activity, indomethacin treatment only partially inhibited cholera toxin mediated secretion while not altering the activation of adenylate cyclase. This difference in response to indomethacin suggests that salmonella and cholera toxin interact differently with the mucosal adenylate cyclase system, i.e. involvement of prostaglandins in the salmonella-mediated but not the cholera toxin-mediated activation of adenylate cyclase (6).

Disadvantages of Chloramphenicol treatment:

The results of 50 cases of typhoid-diarrhoea studied earlier have shown that there was an average drop of haematocrit by 16% from admission haematocrit level during the period of treatment with chloramphenicol in doses of 60 mg/kg/day in 4 divided doses. The dose was decreased to 40mg/kg/day when the temperature was less than 100.F for 24 hours and the treatment continued till 14 days.

There were treatment failures in 3 patients in whom there was no defervescence of fever after 11 days of treatment with chloramphenicol. Two patients relapsed (clinically) approximately between 7-11 days and responded to co-trimoxazole. Defervescence was defined as temperature less or equal to 100 F (rectally) for at least 48 hours. With chloramphenicol treatment in the above dosage, the mean defervescent time was 6.5 days.

ANTIMICROBIAL THERAPY:

Although chloramphenicol is the antibiotic of choice for the treatment of typhoid fever in terminating the febrile toxic course of the disease in the greatest proportion of patients in the shortest period of time (18), Trimethoprim Sulphamethoxazole (TMP) have also proved to be effective in the treatment of typhoid fever (19-23).

Studies by Kamat and Akinkugbe have reported that Trimethoprim Sulphamethoxazole is superior to chloramphenicol with particular reference to therapeutic efficacy and safety. Those treated with TMP suffered less from 'toxic crisis' and had rapid and uniform relief of toxaemia compared to the group treated with chloramphenicol. (22). By using TMP (24) the clinical response with regard to defervescence (mean 2.7 days) and better than chloramphenicol (mean 4.3 days) (22). More relapses were reported in

chloramphenicol treated cases (10%-20%) in comparison to TMP (25). However chloramphenicol clears S. typhi from the bowel much more slowly than co-trimoxazole (around 30 to 60% of cases within one month after start of treatment compared to 85 to 90% with co-trimoxazole). (26).

Some authors suggested that the carrier rate after treatment with TMP may be low because the combination of trimethoprim and sulphamethoxazole is bactericidal, not bacteriostatic like chloramphenicol, and one may therefore expect that carrier will be few. (27). The overall results with chloramphenicol or TMP Trimethopr-m sulphamethoxazole indicate that better treatments should be sought in alleviating the sufferings of patients.

A new cephalosporin drug has been tested in animal models of salmonella infection (28) and in patients with typhoid fever with highly promising results. (29). Ceftriaxone is a new cephalosporin with a broad antibacterial spectrum and an extended half-life of 8 hours (30) and clinical cure rates are generally in excess of 90% (31). In vitro, the MIC of ceftriaxone ranged between 0.03-0.12 mg/l for S. typhi (32). Clinical experience with ceftriaxone in treating meningitis in paediatric patients and other serious bacterial infections in adults (33,34) have encouraged us to examine its clinical efficacy and safety in typhoid fever. As we did not have another treatment group in the previous study, we are not sure whether the blood dyscrasia reported was induced by chloramphenicol therapy. So ceftriaxone against chloramphenicol needs to be evaluated to prove that it is therapeutically superior to chloramphenicol and possess a greater margin of safety.

Hernandez reported ceftriaxone (CTX) is effective against salmonella and because of its long half life its dosage can be once every 12 or 24

hours for 4 days. (29). In this study a rapid clinical improvement was noticed in all 20 cases with negative blood cultures 24 hours after initiation of therapy with no relapses recorded in different dosage scheme. Three different dosage schemes were used: 1.5 gram I/v every 12 hours 3 grams I/v every 24 hours and 4 grams I/v every 24 hours for 4 days.

It is evident from this study that ceftriaxone have the advantage of possibly shortening the clinical course by a week or even less to 4 days, rapid clinical improvement and no occurance of relapses. This would also reduce the occupancy rate of hospital bed that could be utilized for more actuely ill patients and also allow the patient to return quickly to his family or job. Patients compliance will be also ensured by single daily therapy. Side effects, however have been reported and include phlebitis at injection site, rash, leukopenia, thrombocytopenia, eosinophilia, abdominal pain and diarrhoea. (33,34).

The study in Mexico with 20 cases had no control group for comparison. Therefore, more definitive studies are needed now.

3. RATIONALE:

1) About 94% of the typhoid patients present with watery diarrhoea (36) and require intravenous fluid to correct the resulting dehydration. The mechanism of this diarrhoeal phenomenon is still not well understood. Preliminary analysis of the 50 cases of typhoid-diarrhoea showed no elaboration of toxin by S. typhi which was supposed to play a role in the mechanism of diarrhoea. Whether prostaglandins and cyclic-AMP play any role in initiating the fluid secretion in the intestine in typhoid patients is still now known. Further information on this aspect may help to simplify the therapy and shorten the clinical course of typhoid-diarrhoea.

2) Although chloramphenicol is the antibiotic of choice for the treatment of typhoid fever, ceftriaxone is suggested as an alternative because of its promising rapid clinical improvement, single daily therapy and prompt bacteriocidal action.

B. SPECIFIC AIMS:

a) To explore the role of prostaglandin and c-AMP in the pathogenesis of typhoid diarrhoea.

b) To compare the clinical efficacy of chloramphenicol and ceftriaxone in the treatment of typhoid fever.

C. METHODS OF PROCEDURE:

A total of 50 patients will be studied for 2 weeks or more until the subject has clinically recovered from the disease. Patients meeting the following criteria will be admitted in the clinical research unit of I.C.D.D.R. after informed consent.

Criteria for Selection of Patients with Typhoid-Diarrhoea

- Age - 6 months to 60 years
- Sex - Male and Female
- Fever - 101F for 4-20 days
- Diarrhoea - watery diarrhoea without blood and mucous (excluding dysentery)
- Physical signs suggestive of typhoid fever
- Widal - + 'O' titre 100 for adults
  40 below 10 years

Patients not eligible for the Study:

Typhoid cases with complications like perforation, relapse, jaundice and malmutrition.

Hypersensitivity to penicillan and cephalorsporin

Prior treatment with antibiotics

A complete medical history of the onset of illness will be obtained from the patients and/or attendant. Thorough clinical examinations will be performed and investigations will be done on the day of admission for proper management of the case. Assessment of nutritional status will be done by weight, height and age.

During hospitalization daily physical examinations will be done, vital signs and clinical courses recorded and side effects of specific therapy monitored. History recording and physical examination will include:

History:

Fever, chill, rigor, headache, nausea, vomiting, abdominal pain, malaise, joint pain and others

Physical Exam:

Pulse, B.P., respiration, temperature

dehydration status - 8 hourly

C.NS - alertness, delirium, obtunded, stupurous, comatose

Rōse spot - present/absent

Tongue - coated/not coated

Distension of abdomen - present/absent

Tenderness of abdomen - present/absent

Bowel sound - present/absent

Spleen - palpable/not palpable

Liver - palpable/not palpable

Heart - normal/abnormal

Lungs - normal

Presence of added sound - rhonci

- crepitations

Neck rigidity - present/absent

Reflexes - plantar)
) absent/present
knee jerk)

Side Effects of specific therapy of chloramphenicol and ceftriaxone will be looked for both clinically and by laboratory investigations like Hct, platelet and reticulolyte count

Complications:

Gastro-Intestinal bleeding, perforation, pneumonia, urinary tract infection will also be noted and treated appropriately.

TREATMENT:

Patients will be rehydrated with isotonic intravenous fluid according to the degree of dehydration. Oral rehydration solution will not be used because of the chance of abdominal distension and weak general condition of the patient which may not allow the patient to be alert and drink ORS. Patients will be randomly assigned to one of the two treatment groups - Chloramphenicol and ceftriaxone without awaiting the results of investigations.

DOSAGE SCHEDULES:

Chloramphenicol in a dose of 60 mg/kg bodyweight/day in 4 divided doses orally or intravenously will be administered. Intravenous chloramphenicol will be administered in the same dose as oral for toxic patients and in those who will be intubated for measuring C-AMP and prostaglandin. After the patient has become afebrile for 24 hours the dose will be reduced to 40mg/kg/day and will be given orally. Therapy will be continued for a total of 14 days. Ceftriaxone will be given 3 grams I.V. daily for 7 days for adults (>12 years). For children (<12 years) the doses will be 75 mg/kg/day for 7 days. There is no oral preparation available. Both the drugs will be provided by Roche foundation.

The following will be done on the day of admission - 1st day of hospitalization.

ADMISSION:

I. Blood: 6 ml of venous blood will be obtained for performing the following tests:
   1. Total W.B.C. count, differential count, HCT, platelets, reticulocytes
   2. Blood culture
   3. Widal test
   4. Electrolytes, urea, creatinine

II. Stool:
   1. Microscopic examination will be performed according to standard technique of ICDDR,B for W.B.C. and R.B.C. macrophage and parasites
   2. Occult blood test will be done
   3. Stool C/S for S. typhi, Shigella, V. cholerae, enterotoxigenic E. coli, Campylobacter and Rotavirus will be done following standard procedure of ICC.D.D.R.,B

III. Urine:
   1. Analysis will be done from fresh specimens
   2. Culture will be done following standard procedure

The following clinical records will be obtained during study period:

1. Pulse - rate, volume - 8 hourly
2. Rectal temperature - 8 hourly
3. Rate of respiration - 8 hourly
4. Blood pressure - 8 hourly
5. Stool - character, colour, consistency, frequency, quantity by weight - 8 hourly
6. Vomiting - 8 hourly
7. Urine output - 8 hourly
8. Fluid intake quantity - 8 hourly
9. Body weight - daily

The following laboratory tests will be performed as scheduled:

1. Stool culture - on the 1st, 7th and 14th day and 21st days
2. Routine Stool M/E - on 1st, 7th, 14th and 21st days
3. Blood for widal test: 1st and 14th day
4. Blood for culture: 1st day, 3rd day and 14th day
   Blood - C.B.C. - 1st, 3rd, 7th and 14th day
   Platelet count, reticulocyte count 1st, 3rd, 7th and 14th day
5. S. typhi sensitivity to drugs:& MIC: 1st positive isolate
6. Urine culture: 1st day
7. Serum concentration of Chloramphenicol will be measured at 6 hour post dose of chloramphenicol on the 3rd and 7th day of therapy and require 10 to 50 1 of serum. (38). Serum concentration of Ceftriaxone will also be measured at 24 hours post dose on the 3rd, 7th day of therapy (39). Correlation between dose and serum concentration will be monitored during therapy for assurance of adequate and safe dose. (40,41).

Methods of procedure of intubation:

5-10 patients will be selected for intubation studies in typhoid fever. These patients have to fulfil the following criteria:

- adult males
- blood culture positive for S. typhi
- diarrhoea 500 ml/day
- a prehospital history of illness of maximum 7 days
- absence of abdominal distension
- presence of bowel sound

After given informed consent patients will be intubated with a triple lumen polyethylene tube under flouroscopic control. The tube has a rubber bag filled with mercury attached at the distal end to facilitate the passage through the intestines to the ileocecal area, which will approximately tube 24 hours. The 3 meter long tube has an infusion part (I), 15 cm proximal from the proximal collection part (P) and 50 cm proximal from the distal collection part (D). The 15 cm segment between the infusion part (I) and the proximal collection part (P) is the mixing segment. As soon as the distal end has reached the ileocecal or cecal area studies will be performed. Two samples of at least 5 ml each will be collected from P and D for measurements of fasting concentrations of prostaglandins and C-AMP.

Slow-marker perfusion:

A solution of isotone saline containing 300 mg of BSP will be infused through the infusion part at a rate of 0.5 ml/min. After an

equilibration period of 60 minutes 2 samples will be collected from the D-part during two 15 min. periods at a sampling rate of 0.5 ml/min. to calculate the ileocecal flow-rate and prostaglandin and C-AMP concentrations. The ileocecal flow-rates can be calculated with the following formula:

$$\text{pump rate X } \frac{\text{BSP infusion solution}}{\text{BSP distal sample}}$$

Hereafter another perfusion study will be performed. Through the infusion part (I) an isotone saline solution containing 10 g polyethylene glycol (PEG) will be infused at a rate of 10 ml/min.

After an equilibration period of 60 minutes. For adequate mixing in the mixing segment, samples will be collected from both collection parts during three 15 minute periods at a rate of 0.5 ml/min., with a staggering period of 15 minutes between the P-part and the D-part. This triple-lumen perfusion study will allow us to calculate net transport of water and ions in the distal ileal segment and to measure production rates of prostaglandins and C-AMP in this test segment.

Samples drawn from ileum will be tested at 24 hours of admission and the test repeated during convalescent, 3 weeks after admission. Ileal fluid collected will be tested for C-AMP, Prostaglandin PGE_2, Protein and Electrolytes, W.B.C., Quantitative culture for Salmonella.

Catheter stool for C-AMP will be taken before initiation of drug. For estimation of C-AMP, stool and illeal samples will be collected and preserved in 3 volumes of 20% trichloroacetic acid (TCA) in the refrigerator prior to isolation and estimation of C-AMP. TCA will prevent degradation of C-AMP by phosphodiesterase present in samples. Estimation of C-AMP in the sample will be done by a protein-binding competition method using a reagent kit BDH chemicals. (37).

D. SIGNIFICANCE:

The results of this study will generate new knowledge regarding the mechanism of diarrhoea in typhoid fever. This will help in developing a better management of the disease and thereby reduce the sufferings of the patients.

E. FACILITIES REQUIRED:

1. Office space - The present office space will be utilized
2. Laboratory space - ICDDR,B assisting lab space will be used
3. Hospital Resource - Clinical Research ward space will be required
4. Animal Resources - Will be used
5. Logistic support - Data processing

F. COLLABORATIVE ARRANGEMENTS:

Collaborative arrangements will be made between I.C.D.D.R.B. and Dr. J. Rask-Madsen in Denmark for performing the radioimmunological measurements (RIA) of Prostaglandin E_2. Similar procedures will be followed to obtain sample of PGE_2 as has been practised for the protocol entitled "Role of endogenous prostaglandins in secretory diarrhoeas", by Speelman et al from I.C.D.D.R.B.

G. DATA ANALYSIS:

1. Means ± S.D. of quantitative analysis of stool
2. Frequency of positive cultures of blood, stool and urine
3. Means ± S.D. of fever, diarrhoea, positive stool culture
4. Variation in clinical picture according to age and sex
5. Comparing means + S.D. of frequency of diarrhoea in patients with *S. typhi* alone in stool and also those with other pathogens
6. As the study cannot be double blinded so to minimize errors due to observer bias, measurements of temperature, clinical condition and stool output will be made with extreme care and objectivity. Subjective features will not be emphasized.

   Response to therapy will be evaluated by comparing the following parameters in two groups of patients:

   a) Time of defervescence - Defervescence defined as temperature less or equal to $100^{o}F$ (rectally) for at least 48 hours
   b) Treatment failure - Patients who will have pyrexia for more than 7 days and show no improvement despite therapy
   c) Relapses - Return of identical clinical symptoms and signs of typhoid fever as of admission
   d) Changes in the Hct, Platelet, Reticulocyte count on admission, 7th, 14th day of treatment
   e) Day of blood culture negative

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ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

Diarrhoea is a common cause of childhood morbity and mortality and typhoid diarrhoea is more in children than adult. So children and adults will be required for this study. This study will enable us to gain knowledge for better management of diarrhoea in future.

There is no risk for the patient except venous blood drawn for performing routine investigations in diagnosing and treating the case appropriately. This is a standard procedure carried out with aseptic measure. There is no alternative method and likelihood of any risk. To understand the role of prostaglandin and C-AMP in the pathogenesis of diarrhoea in typhoid, 5-10 patients will be intubated to collect ileal fluid for prostaglandin E_2 and C-AMP isolation. These patients will be intubated only when the criteria described in the text will be met and hence will be avoiding the risk of perforation.

Daily follow-up of patients both clinically and by laboratory investigation treatment of the disease will be prompt and sufferings of the patients reduced. The chance of death due to development S. typhi septaecaemia will be minimized.

Confidentially of the date will be mentioned by using code numbers and patient numbers rather than specific name. Data will be safeguarded by keeping it under lock.

Signed consent will be taken from the patients or guardians after explaining the purpose and procedure of the study. Compensation is not required. Proper medical care will be provided and diet supplied.

There is an interview of the patient when recording medical history of his illness. This will be acceptable to the patient and will also help in managing the case. Ten minutes interview will be taken.

../2

Patients will be directly benefited from this study by specific automicrobial drug after thorough laboratory investigations and proper care taken by investigators. Proper management of the patient will reduce the suffering of the patient and his family members. The benefit to the society will be to a great extent by improvement in the management of disease which will be better understood by doing this research. The treatment given will reduce the carrier rate and reduce the contamination of the environment. New cephalosporin will shorten the course of the disease and improve clinical cure within 7 days or less. Specific care and early identification of this serious disease will help the patient more than the inconvenience of being hospitalized.

3. The study will require the use of patient records, blood, stool, urine and small intestinal fluid from 5-10 patients. Fetus and abortus samples will not be used.

DETAILED BUDGET:

1. Personnel Service:

Name	Position	% of Effect	Project Requirement Taka	Dollar
Dr. Asma Khanam	Principal Investigator	30%	37,915	
Dr. T. Butler	Co-Investigator	10%		6,700
Dr. Speelman	"	5%		1,850
Dr. Samir	"	25%	16,885	
Dr. N. Hoque	"	10%	7,600	
Dr. I. Kabir	"	10%	9,420	
Dr. Hamidur Rahman	"	5%	3,800	
Senior Staff Nurse	3 x 10	3 x 10	13,270	
Aid Nurse		2 x 5	2,605	
1 Sr. Lab. Tech (Clinical Pathology)		20%	6,440	
1 Statistician		5%	3,500	
Dr. S.K. Roy		5%	4,710	
			106,145	US 8,550

2. Supplies and Materials:

(a) Clinical supplies, needles, gloves, syringe, testube etc. US $ 500

(b) Lab test

Blood:	Cost:	Total:
C.B.C. - 3	Tk. 4.20	Tk. 630
Widal - 2	Tk. 29.00	Tk.1450
Culture - 2	Tk. 15.00	Tk.1500
Electrolytes - 2	Tk. 40.00	Tk.4000
		Tk. 7580

Stool:	Cost Tk.	Total
Microscopy - 3	2.50	350.00
Culture - 3	15.00	2250.00
Electrolyte with protein - 2	11.00	1100.00
Quantitative S. typhi culture - 1	10.00	500.00
Elisa - 1	50.00 x 30	1500.00
Urine:		
Analysis - 2	3.00	300.00
Culture - 1	7.50	375.00
C-AMP		
P.G.		13955.00
Toxin assay:		
LT, ST test - 1	30.00 x 50	1500.00
	Total Tk.	15455.00
Stationaries		3000.00
Medicine		15000.00
3. Equipment:	- Nil	
4. Patient hospitalization	-50 x 14 x Tk.150	105000.00
5. One patient care	- Nil	
6. ICDDR,B Transport -	20 x 15 x 4.50	1350.00
7. Travel & Transportation of persons:	Attending International Seminar on Typhoid fever	US $ 2000.00
8. Travel & Transportation of things	- Nil	
9. Printing and Reproduction		US $ 500.00
10. Other contractual services	- Nil	
11. Construction, Renovation, Alteration	- Nil	

B. BUDGET SUMMARY:

		Taka	Dollar
1.	Personnel Services	106,145	8,550
2.	Supplies and Materials, Medicine, Blood	19,350	500
3.	Patient hospitalization	105,000	-
4.	Travel and Transportation	-	2,000
5.	Rent, Communication, Utilities	100	-
6.	Printing and Reproduction	-	500
	Total Tk.	230,595	US $11,550

(1 US $ = Tk. 25)	=	US $ 9225
Total US $	=	20775
Incremental cost 10%	=	2077
Grand Total	=	US $22852

CONSENT FORM

TYPHOID - DIARRHOEA STUDY

Typhoid fever is a sickness which we believe is familiar to you. In the past people used to die or suffer for days or years due to its complications. At ICDDR,B we encounter patients presenting typhoid fever with diarrhoea. Although chloramphenicol helped the physicians to treat the disease effectively and reduce the mortality, better drugs are sought for shortening the duration of treatment and rapid clinical improvement and less toxicity. Ceftriaxone has been tried abroad with very promising results. The fever comes down quickly and hospital stay is shorter. So we would like to study the clinical efficacy with the standard drug, chloramphenicol and a new drug ceftriaxone having activity against tyhpoid germs. Chloramphenicol will be given orally or intravenously according to your disease condition, 4 times a day for 14 days and ceftriaxone single dose daily for 7 days by intravenous route as there is no oral preparation. You will have to stay for 14 days for your treatment and follow up of any untowards side-effect of the drug.

Your treatment. By this time 6 ml of blood will be drawn for culture, widal test, electrolyte and complete blood count 1st, 3rd, 7th and 14th day of hospitalization for diagnosis, clinical assessment and evaluation of therapy. Besides this stool and urine samples will be examined routinely.

All records of your treatment in the hospital will be kept confidential.

You will be given usual treatment even if you do not participate in the study. If you agree to participate in the study please sign here.

_____ _____

Signature of the Investigator | Signature or L.T.I. of patient or guardian

Witness: | Date: _____

1. _____ | Case No. _____

সম্মতি পত্র

টাইফয়েড -ডাইরিয়া স্টাডি

টাইফয়েড জ্বর সম্বন্ধে আপনারা নিশ্চয়ই অবগত আছেন। অতীতে বহু লোক এই রোগে মারা যেত। আমরা আই, সি, ডি, ডি, আর, বি-র হাসপাতালে টাইফয়েড জ্বরের সংগে পাতলা পায়খানা সহ অনেক রোগীর চিকিৎসা করে থাকি। যদিও ক্লোরমফেনিকল ঔষধ দ্বারা চিকিৎসা করে অনেক রোগীকে মৃত্যুর হাত থেকে বাঁচানো যায় তবুও আমরা নতুন কোন ঔষধ দ্বারা কি করে কম সময়ে রোগীকে সারিয়ে তোলা যায় তার জন্য চেষ্টা চালিয়ে যাচ্ছি। সেফট্রায়াকসন নামে একটা নতুন ঔষধ টাইফয়েড জ্বরে চিকিৎসা দিয়ে বিদেশে বেশ ভাল ফল পাওয়া গেছে। এই ঔষধ ব্যবহারে টাইফয়েড রোগীর জ্বর এবং অন্যান্য উপসর্গ খুব তাড়াতাড়ি সেরে যায়। অতএব আমরা আপনাকে/আপনার রোগীকে ক্লোরামফেনিকল শিরায় অথবা মুখে দিয়ে চার বার ১৪ দিন অথবা সেফট্রায়াকসন দিনে একবার শিরায় সাত দিন দিয়ে চিকিৎসা করাবো এবং দুটো ঔষধের গুনাগুন পরীক্ষা করবো। চিকিৎসার জন্য আপনাকে ১৪ দিন হাসপাতালে থাকতে হবে।

হাসপাতালে থাকার সময় আপনার/আপনার রোগীর রোগ নির্ণয় এবং অবস্থার উন্নতি জানার জন্য ১ম, ৩য়, ৭ম এবং ১৪তম দিনে ৬ মিলিলিটার রক্ত নিয়ে পরীক্ষা করবো। এছাড়াও আপনার/আপনার রোগীর পায়খানা এবং প্রস্রাব পরীক্ষা করা হবে।

আপনার/আপনার রোগীর যাবতীয় কাগজপত্র গোপন রাখা হবে।

আপনি যদি গবেষণায় অংশগ্রহণ করতে রাজী নাও থাকেন কিংবা কোন কারণে গবেষণা পরিত্যাগ করতে চান, তবুও হাসপাতালের সাধারণ সুচিকিৎসা আপনাকে দেওয়া হবে। আপনি রাজী থাকলে নীচে সই করুন।

গবেষকের স্বাক্ষর

রোগীর/অভিভাবকের স্বাক্ষর/টিপ সহি

তারিখ----------

CONSENT FORM FOR INTUBATION

TYPHOID-DIARRHOEA STUDY

We want to study typhoid fever with diarrhoea. We want to study the mechanism of diarrhoea in patients with typhoid fever which will contribute towards the improvement in the management of typhoid fever. For this, we will introduce a thin rubber tube which will pass through your stomach to the small intestine and be kept there for 24 hours. Intestinal fluid (10 ml) will be drawn through this tube and measured for C-AMP, PGE_2 which are thought to play a role in the production of diarrhoea in patients with typhoid fever. During this period you will not be allowed to take any food except water. You will be treated with specific antibiotic and intravenous saline. The same procedure will be repeated after 3 weeks. Your normal treatment will continue whether you agree to participate in the study or not. If you agree, please sign here. Thank you.

Signature of the Investigator

Signature of the patient/
Thumb print

নল প্রবেশ করার সম্মতি পত্র

আমরা টাইফয়েড জ্বরে ডাইরিয়ার কারণ সম্বন্ধে জানতে আগ্রহী। এর ফলে টাইফয়েড জ্বরের চিকিৎসার বিশেষ অগ্রগতি হবে। আমরা আপনাকে বা আপনার রোগীকে একটি অতি চিকন রাবারের টিউব পাকস্থলীর মধ্য দিয়ে ক্ষুদ্রান্ত্রে প্রবেশ করিয়ে ২৪ ঘন্টা রাখব। এই সময়ে এই নল দিয়ে ক্ষুদ্রান্ত্রের নিঃসৃত রস সংগ্রহ করব যার মধ্যে c-AMP, PGE_2 নির্ণয় করব। এই সকল জিনিস টাইফয়েড ডায়রিয়ায় বিশেষ গুরুত্বপূর্ণ ভূমিকা পালন করে। এই সময়ে তাহাকে পানি ছাড়া অন্য কিছু খেতে দেওয়া হবে না। এই সময়ে আপনি ঔষধ এবং শিরার স্যালাইন পাবেন। একই ভাবে তিন সপ্তাহ পরে আবার পরীক্ষা করা হবে।

গবেষকের স্বাক্ষর

রোগীর/অভিভাবকের স্বাক্ষর/টিপ সহি

তারিখ----------