Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date August 10, 1987
August 16, 8
2C

Principal Investigator Dr. FPL Van Loon

Application No. 87-021

Title of Study The role of endogenous prostaglandins in secretory diarrhoea

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B) WHO

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 N.A.
6. Will precautions be taken to protect anonymity of subjects — ✓Yes No
7. Check documents being submitted herewith to Committee:
   - NA 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
   - NA 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.

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

(PTO)

Principal Investigator

10.8.87.

OCT 28 1987

Trainee

87-021
16.08.87

SECTION 1 - RESEARCH PROTOCOL

1. Title: The role of endogenous prostaglandins in secretory diarrhoeas

2. Principal Investigators: Dr. F.P.L. Van Loon (Dhaka)
Dr. I. Kabir (Dhaka)
Dr. J. Rask-Madsen (Denmark)
Dr. K. Bukhave (Denmark)

3. Starting Date: 1 October 1987

4. Completion Date: 1 April 1988

5. Total Incremental Cost: US $1,800

6. Scientific Programme:

This protocol has been approved by the Clinical Sciences Division.

Signature of Programme Head

Date: 10.8.87.

7. Abstract Summary

Our study "Role of endogenous prostaglandins in secretory diarrhoea" (ICDDR,B Protocol No. 83-049) has met with unforseen problems. (i) The design was to use intestinal perfusions at two different rates - 0.5 ml/min, also called "slow marker" and 10 ml/min, "steady state".

It now appears that the endogenous prostaglandins show such a variability in the "slow marker" group of patients that the results are to be considered as without value. (According to Dr. Rask-Madsen from Denmark, co-principal investigator, and in charge of the biochemical analysis.) (ii) In many patients, the perfusions had to be interrupted and the whole procedure to be considered as valueless because of the frequent power failures which occurred during the study period. (iii) The results of the "steady state" perfusion are quite promising,but the number of patients is too low. To quote Dr. Rask-Madsen: "Due to initial technical difficulties we have only six acute and four convalescent patients providing valid results (see Table)...it seems necessary to carry out studies in another five patients with a perfusion speed of 10 ml/min." The considerable variability of the figures (See SEM in table) explains the necessity for a further study."

Table: Preliminary results

	Net fluid transfer ml/cm x h	PGE* ng/min	Purging Rate 1/8 h
Acute (n=6)	+4.7+1.5	2.5+0.7	7.5+1.0
Acute + indomethacin (n=6)	+0.3+1.2	1.4+0.4	4.0+1.2
Convalescence (n=4)	-0.7+0.5	0.6+0.2	

*Other PGs not yet known.
Mean +SEM. - denotes absorption, + denotes secretion.

The aims of the present protocol are (i) to complete what has been a technically difficult but certainly interesting study, to (ii) determine whether in cholera patients indomethacin indeed causes a striking reduction of the net fluid transfer in the intestine, the purging rate and the PGE concentration, as the results already available indicate. We intend to study ten (10) patients with acute cholera using the steady-state perfusion technique, a procedure already frequently used at ICDDR,B. In the fluid aspirated from the intestine, we will measure levels of all endogenous prostaglandins (PGs) (PGE_2 PGE_1 , PGF_2 alfa, 6 keto PGF_1 and tromboxane) as well as 5 hydroxytryptamine.

We will further study the effect of a bolus injection of indomethacin (1.0 mg/kg) on these variables. The patients

studied will be requested to return to the hospital two weeks after discharge for intubation and control perfusion studies without administration of the drug.

We propose to study 10 patients to be certain that valid results will be obtained in at least five, the minimum number required. Additional valid results will contribute to further strengthen the value of the results.

This protocol is meant to complete the ICDDR,B protocol 83-049.

8. **Reviewers:**

(a) Research involving human subject: _____

(b) Research Review Committee: _____

(c) Director: _____

N.B. This research protocol is a part of a collaborative researh project with Dr. J. Rask-Madsen from Denmark. Funds for this study will be requested from WHO.

A. INTRODUCTION

1. Objectives:

a. To measure the endogenous prostaglandins (PGs) and 5-HT in jejunal fluid of patients with cholera during the acute phase and in convalescence.

b. To study the effect of indomethacin, a PG-synthesis inhibitor, on endogenous prostaglandin production, and secretion of fluids amd electrolytes.

2. Background

The role of the arachidonic acid (AA) - prostaglandins (PGs) system in the regulation of intestinal ion transport has not yet been fully established, but there is now ample evidence to suggest that they may be part of a complex regulatory mechanism (1,2).

PGs are synthetized throughout the gastrointestinal tract where different regions are characterized by different profiles of AA-metabolizing enzymes (3). Hormones, paracrine mediators, and neurotransmitters interact with specific surface receptors which in turn initiate the enzymatic release of AA from the phospholipid pool (4). The released AA can then be oxygenated via the cyclooxygenase pathway to the unstable endoperoxides which, dependent on the specific cell, are further converted to PGE_2 and $PGF_{2\alpha}$ the major products on the intestinal mucosal cell - as well as prostacyclin (PGI) and thromboxane (TX). Alternatively, AA may be oxygenated via the lipooxygenase pathway which leads to the formation of leukotrienes (LTs) - substances which have recently been discovered in leukocytes, appear to play an important role in chemotaxis and immune response (5), and may be involved in diarrhoea caused by invasive organisms that is accompanied by inflammation, as well as in chronic inflammatory bowel disease.

The cyclooxygenase pathway is blocked by nonsteroid anti inflammatory compounds like indomethacin and aspirin. The

lipooxygenase pathway is not affected by cyclooxygenase inhibitors, but the transformation of LTA_4 to LTB_4 appears to be inhibited by 5-amino-salicyclic acid (5-ASA), the active moiety of sulphasalazine. In contrast, glucocorticoide act by reducing the supply of substrate for PG/LT biosynthesis due to activation of peptide phospholipase inhibitors, provisionally named macrocortin or lipomodulin (6).

PGs are rapidly metabolized with no evidence of storage, and their release is considered to reflect de novo synthesis. Thus PGs appear to have paracrine actions and may be regarded as local regulators, rather than circulating hormones - more than 90% being inactivated during a single passage through the lungs (7).

In the gastrointestinal tract PGs are considered to play a role in the control of motor activity and have been implicated as determinants of secretion (8,9). Thus PGs have been known to cause diarrhoea in humans since it was incidentally observed in 1970 that women receiving PG infusions for termination of pregnancy, frequently developed diarrhoea as an adverse effect (10). Initially this effect was attributed to changes in motility, but later it was shown that the copious watery diarrhoea following parenteral, oral, and jejunal administration of PGs had the characteristics of secretory diarrhoea (4,8,11).

Previous in vitro studies have been handicapped by the inability of isolated intestinal mucosa to respond to PG

concentrations which may be considered physiological. However, recent studies by Bukhave and Rask-Madsen (11) have demonstrated that secretory responses to low (physiological) doses (10^{-11} - 10^{-7} M) of PGE_2 can be obtained in Ussing chamber preparations of human jejunal mucosa, provided that the production of endogenous PGs is blocked by indomethacin. These studies convincingly illustrated that the inability of untreated tissues to respond to so-called "physiological" concentrations of exogenous PGE_2 is caused by preformed PGE since the in vitro formation of endogenous PGE_2 by untreated tissues equalled the threshold concentration for effect of exogenous PGE_2 in the same tissue. Furthermore, pre-treatment of the tissue with indomethacin practically abolished PGE_2 formation in vitro, at the same time increasing its sensitivity to exogenous PGE_2. PGs may have pathophysiological effects (9, 12), in addition to pharmacological (6, 13) and physiological ones (11), on intestinal ion transport. However, the primary obstacle for establishing the pathophysiological role of PGs in secretion is the artificial invitro production of PGs by aggregating platelets, which occur spontaneously with blood sampling, or by tissue specimen as a result of mechanical damage by the biopsy forceps. These events cannot be controlled by the addition of PG synthesis inhibitors or anticoagulants to the test tube (14, 15). On the other hand determination of PG-metabolites in plasma or urine would at best reflect the total body production (15).

Considering these methodological problems, data on the amount of AA metabolites released into the gastrointestinal fluid presently appear to provide the most reliable index of the balance between gastrointestinal PG synthesis and degradion in vivo (12, 16-22). This approach is also attractive because it permits estimation of parent PGs and their metabolites in parallel, as well as specific stimulation by luminal and neurohumoral secretagogues, in addition to non-specific stimulation due to hypoxia and chemical or physical damage in vivo.

Using the above mentioned approach Rask-Madsen et al. have shown abnormally high concentrations of PGs in the intestinal lumen in:

1. The irradiation syndrome following physical damage to the epithelial membranes (4).
2. Collagenous colitis, maybe due to hypoxia caused by a diffusional barrier associated with subepithelial deposits of collagen (19).
3. Fluid-discharging villous adenoma of the rectum, maybe as a result of the neoplasia per se (12), or maybe hypoxia due to low vascularization of the tumour epithelium.
4. Malignant carcinoid syndrome (21), probably in response to high circulating levels of 5-HT (4).

5. "Nervous diarrhoea", as observed in certain patients classified as irritable bowel syndrome, may be due to increased parasympathetic influence (17) or possibly specific food intolerance (22).
6. Coeliac disease, as a consequence of crypt hyperplasia (4) with increased local release of 5-HT (23), because PGE_2 levels are significantly raised even in the absence of active inflammation (20).
7. Inflammatory bowel disease primarily due to release of PGs from infiltrating leukocytes (24).

Analysis of the data obtained in these above various clinical conditions shows that a positive correlation exists between luminal PGE_2 and stool volume in patients with secretory diarrhoea (25). On the other hand, normal PGE_2 levels were found in patients not responding to indomethacin, e.g. Verner Morrison's syndrome (cAMP being the second messenger) and disaccharidase intolerance (osmotic diarrhoea). In contrast, the elevated luminal PGE_2 levels found in patients with inflammatory bowel disease correlate primarily with the severity of mucosal inflammation, in agreement with the observation that treatment with potent cyclooxygenase inhibitors decreases absorption and increases the relapse rate (26) - probably by a diversion of AA metabolism via the lipooxygenase pathway.

The effects caused by PGEs are in many respects similar to those caused by cholera toxin. Both PGE in pharmacological doses and cholera toxin increase intestinal cAMP by stimulating the intestinal adenylate cyclase activity (27,28). It has been speculated that PGs might be mediators of the secretory effects of cholera toxin (29). The use of PG synthesis inhibitors has been reported to decrease or inhibit the secretory effects of cholera toxin in vitro (30, 31), but it has been generally accepted that the mechanism by which PGs elicit secretion depends on cAMP and that the role of PGs is secondary rather than primary (32-33).

However, the above mentioned studies on stripped human jejunal mucosa showed evidence of secretory effects that could be obtained with PG concentration 100-1000 times lower than those required to affect the adenylate cyclase activity, provided that the in vitro formation was suppressed by indomethacin (11). Furthermore, recent observations suggest that intramural nervous reflexes play a role in secretion induced by cholera toxin and dihydroxy bile acids, since these secretaggues in experimental animals - besides activating the adenylate cyclase activity - trigger the release of 5-HT (34), a substance that is postulated to activate phospholipases in the cell membrane, hydrolyze phospholipids, and lead to the formation of AA and its metabolites (4, 25).

Since intestinal secretion is induced by 5-HT and cholinergic agonists (35), both being neurotransmitters which stimulate PG synthesis and raise intracellular Ca without affecting cAMP, a revised view for the cause of secretion would be that PGs act by increasing the gating of Ca across the serosal cell membrane and cAMP acts by releasing intracellular reservoir Ca (25). Consequently, both PGs and cAMP may be considered "true" second messengers for the stimulus-secretion coupling via intracellular free Ca, as illustrated in the model of postulated intracellular control mechanisms adapted from Powell and Field (35), but modified by indicating that PG formation occurs in response to secretagogues like 5-HT (25).

This hypothesis on the mechanisms involved in secretory diarrhoea has been further substantiated by recent studies on the mechanism of diarrhoea in a patient with carcinoid syndrome (21) and following withdrawal of morphine from morphine tolerant rats (36). In the carcinoid syndrome PGE_2 levels in the jejunal fluids were markedly increased, but both indomethacin and ketanserin reduced the diarrhoeal volume and the local intestinal PGE_2 concentrations. In morphine tolerant rats naloxone-induced morphine withdrawal reversed fluid absorption to secretion without changing mucosal cAMP levels, but markedly enhanced PGE_2- and 5-HT release. Indomethacin prevented withdrawal-induced fluid

secretion and the increase in PGE_2 release. In contrast, ketanserin prevented secretion without influencing the release of 5-HT. Also the a_2- receptor agonist, clonidine, promoted absorption during withdrawal, whereas atropin failed to influence fluid transport (36).

Previous work:

Preliminary studies including our own (see table p.3) have demonstrated that PGE_2 levels in jejunal fluids of patients with cholera were significantly raised and were negatively correlated to the time following onset of diarrhoea. Jejunal flow rates of PGE_2 were also significantly raised and positively correlated to the stool output during the acute phase of the disease. These reults suggest an important role of PGs, in addition to cAMP, in human cholera (37).

3. Rationale

The data as summarized under "Background" and "Previous work" form the rationale for further studies.

3.1 Both PGE_2 and cholera-toxin (CT) increase intestinal cAMP, and PGE_2 is elevated in cholera. It has therefore been speculated that PGs might be mediators of the secretory effect of CT. So far it has been accepted that PGs elicit secretion via the cAMP pathway, yet PGs can elicit intestinal secretion in concentrations 100-1000 times lower than required to affect the adenylate cyclase activity. PGs might thus be second messengers in their own right.

3.2 Another argument to reconsider the role of PGs is the fact that CT has been shown to trigger enterochromaffin cells to release 5-HT. 5-HT, a neurotransmitter that induces intestinal secretion, stimulates PG synthesis and

raises intracellular Ca without affecting cAMP. These two arguments combined with the exciting results of our first study form the rationale for the present one.

Confirmation of the results obtained to date is necessary lest we loose the benefits of a potentially most interesting research effort.

B. SPECIFIC AIMS: See objectives

Patient selection:

We intend to study adult patients, male and female, presenting to ICDDR,B Treatment Centre with a history of acute watery diarrhoea for less than 24 hours. Patients should be at least moderately dehydrated. Only those patients with an initial purging rate of at least 200 ml/hour, and no prior medication will be eligible for this study. Fresh faecal specimens will be examined by darkfield microscopy for the presence of V. cholerae and be sent for culture. The study will be explained to the patients by one of us (Dr. Kabir) and the patient will be invited to participate in the study. Provided as informed written consent has been obtained the patient will be transferred to the study ward. A complete physical examination will be done and rehydration will be performed with intravenous fluid. No oral rehydration solution will be used during the study period.

Perfusion studies:

Patients will undergo jejunal intubation by an oral or nasogastric triple-lumen tube. Preferably, the intubation will be carried out in the morning, the patient being in a fasting state. The position of the tube (distal aspiration port 20 cm distal to the ligament of Treitz) will be checked under fluoroscopy. Ten ml of jejunal fluid will be aspirated for determination of fasting concentrations of PGs and 5-HT.

Subsequently a "steady state" perfusion (42,43) of the jejunum segment will be performed, using BSP as a non-absorable marker. The equilibration period during the "steady state" perfusion will be 90 minutes with an infusion rate of ± 10 ml/min. After the equilibration period, 4 sequential collections of 10 ml at 30 minutes interval will be used for determination of transport rates of fluid, Na, Cl, K, PGs and 5-HT. Hereafter, the response to a bolus injection of indomethacin (1.0 mg/kg) will be studied by collecting samples at 30, 60 and 90 minutes.

Indomethacin I.V. in this dose has been used before by Thornell et al. (45). The treated 20 patients with confirmed gallbladder or biliary pain. Pain was relieved within 30 minutes in all 24 treatments. Apart from some vertigo and slight nausea there were no side effects. In our own study,

no side effects were observed, except for slight abdominal discomfort and dizziness during about 5 minutes in a few patients.

All patients studied will be requested to return to the hospital two weeks after discharge for jejunal intubation, sampling of jejunal fluid, and control perfusion studies without administration of drugs.

In summary, 10 patients will be investigated twice, once during acute cholera and once during convalescence. The level of PGs and 5-HT in the jejunal fluid will be determined, and the net intestinal fluid transfer will be calculated, both before and after the administration of indomethacin (the latter only during the acute cholera phase).

Laboratory analyses:

PG and 5-HT measurements:

Internal standards will be added to the biological samples at the biochemical laboratory at ICDDR,B prior to the preliminary extraction, in order to correct for losses of unstable PGs during storage and transport.
Radioimmunological measurements (RIA) will be performed for determination of PGE_2 , PGE_{2a} , 6 keto PGF_{1a}, and TXB_2 in jejunal fluids aspirated before and during "steady state" perfusion of the small intestine. PGE_2 is considered to be the PG responsible for ion secretion, whereas PGF alfa and 6 keto PGF alfa are used as indices of basal PG production in

epithelial and endothelial cells, respectively. TXB_2, which originates primarily in platelets, is used as marker of vessel injury with bleeding. Determination of these PGs, which include purification by extraction and column chromatography before the quantifications are performed by RIA (17), are currently carried out in the Danish laboratory. The RIAs for PGE_2 and PGF_{2a} were recently checked by quantitative gas chromatography-mass spectrometry (48).

Determination of 5-HT will be performed by HPLC according to Sperk (50). The samples will be mixed immediately with cold perchloric acid (final concentration 0.2 M) and ascorbic acid (final concentration 0.01 mM).

Data Analysis

The data will be analysed using relevant standard parametrical statistical methods, such as the Student's t-test for paired and nonpaired variates and the analysis of variance or non-parametrical statistical analyses - preferably Wilcoxon's test for paired variates and Mann Withney's U-test. Subjects will serve as their own controls.

D. SIGNIFICANCE

Definition of the role of PGs in intestinal secretion in patients with diarrhoea may provide a rationale for the

clinical use of potential anti-diarrhoeal drugs that inhibit PG metabolism or interfere with the action of secretagogues on arachidonic acid metabolism.

E. FACILITIES REQUIRED

No new facilities required.

F. COLLABORATIVE ARRANGEMENTS

This protocol is a part of a collaborative research project between ICDDR,B and Dr. J. Rask-Madsen in Denmark.

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ABSTRACT SUMMARY:

1. Adult patients with diarrhoea caused by cholera form the subject population.
2. Introduction of a double or triple lumen tube till beyond the ligament of Treitz does not bear any substantial risk. No problems or complications are known. No complications were encountered in previous studies. Perfusion of the small intestine for a few hours with a non absorbable marker as sodium sulfobromphtalein (BSP) is without any risk. Indomethacin, is safe, which has already been used for years, and has limited, well known side effects (and will not be used in patients with a history of ulcer disease.
3. The procedures are carried out by qualified and experienced doctors; it is highly unlikely that any complication will occur.
4. Data collection sheets will be kept in a locked place. If published, data will show no reference to the identity of the patient.
5. Informed consent (signed or thumb printed) will be obtained from the patients at the time of admission into the study.
6. Does not apply.
7. Direct benefit to the patient will be the cost free treatment

of the diarrhoeal episode. Society in general may benefit in the future of the development of new antisecretory drugs which act through inhibition of prostaglandin synthesis.

8. In this study we will use the normal hospital charts and we will collect fluid from the jejunum through aspiration.

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

CONSENT FORM: PG-STUDY II

You have cholera which provokes a very important loss of water from your body and requires replacement of the lost water by I.V. fluids. We want to study your intestinal fluid to see how to help more efficiently cholera patients in the future. Therefore we want to introduce a small tube through your mouth or nose to the intestine to collect this fluid. This procedure will take about half a day. Hereafter the tube will be removed. This procedure is completely safe but may cause some discomfort in the nose or throat.

We will request you to come back to the hospital 2 weeks after discharge. If you come back, we will reimburse your travel expenses and a daily income.

If you do not want to be included in this study, you will not be penalized in any way but you will receive the same proper treatment in the hospital. You may also decide to withdraw from the study at any time.

If you accept to join the study, please sign the consent form here below.

Signature of patient or thumb impression

Signature of the investigator Date:

SECTION III

BUDGET

				Project Req.	
1.	Personnel Services				
	Name	Designation	% time	Tk	US$
	Dr FPL Van Loon	PI	30		
	Dr I Kabir	CO-I	10	3100	
	Senior nurses (3)		10	9500	
	Cleaners (3)		10	3000	
	Clerk		10	2800	
	Secretary		10	2000	
2.	Laboratory Expenses				
	Darkfield Stool cultures Chemicals				70
3.	Equipment				420
4.	Pt. hospitalisation	12x3x150		4500	
5.	Outpatient	10x100		1000	
6.	Transport				
7.	Transport of samples				400
8.	Printing,Pub. & Reproduction				100
Total				24000	990

Grand Total - US 1,767

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GPO Box 128 Dhaka-2 Bangladesh

আন্তর্জাতিক উদরাময় রোগ গবেষণা কেন্দ্র
(আই, সি, ডি, ডি, আর, বি,)

সম্মতি পত্র

(সি, জি, ষ্টাডি-২)

আপনার কলেরা রোগ হয়েছে। এই রোগে প্রচুর পরিমানে জলীয় পদার্থ ও লবণ বের হয়েছে। সেই জন্য আমরা আপনার শিরায় স্যালাইন প্রবেশ করিয়ে চিকিৎসা করবো। আমরা আপনার কুদ্রান্ত্রের জলীয় পদার্থ পরীক্ষা করবো। এই পরীক্ষার জন্য আপনাকে একটা সরু রবারের নল গিলে ফেলতে হবে এই পরীক্ষা প্রায় অর্ধদিবস চলবে। এবং এর পর রবারের নলটি বের করে ফেলা হবে। এই পরীক্ষা সম্পূর্ণ নিরাপদ, কিন্তু আপনি এবং গলায় সামান্য অসুবিধা বোধ করতে পারেন।

আপনাকে ২ সপ্তাহ পর আবার হাসপাতালে আসতে অনুরোধ করা হচ্ছে। সে সময় আমরা আপনাকে যাতায়ত ও পারিশ্রমিক বাবদ কিছু টাকা দেব। আপনি এই গবেষণায় অংশগ্রহণ না করলে, কিংবা গবেষণা চলাকালীন হটাৎ পরিত্যাগ করলেও আপনাকে কলেরার চিকিৎসা দেওয়া হবে। আপনি রাজী থাকলে নীচে সই করুন।

গবেষকের স্বাক্ষর

রোগীর স্বাক্ষর/টিপ সহি

তারিখ----------