hment 1. Date 14.8.81

ETHICAL REVIEW COMMITTEE, ICDDR,B.

ipal Investigator Dr. L. Gothefors Trainee Investigator (if any) _____

cation No. 81-036 Supporting Agency (if Non-ICDDR,B) _____

of Study "LOCAL IMMUNITY AND URAL" TERMINATION OF CHOLERA Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

e 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)

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

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:
   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.

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

Asma Khanam for Dr. L. Gothefors
Principal Investigator

Trainee

81-036
Rec'd 14/9/81

SECTION I - RESEARCH PROTOCOL

Title:	LOCAL IMMUNITY AND "NATURAL" TERMINATION OF ACUTE CHOLERA
Principal Investigator:	Dr. L. Gothefors
Co-Investigators:	Dr. A. Khanam, Dr. G.N. Tahbeat, Dr. D. Sack, and Dr. J. Holmgren
Starting Date:	1 October 1981
Completion Date:	30 September 1982
Total Direct Cost:	US$ 28,250

Scientific Program Head:

This protocol has been approved by the Pathogenesis & Therapy Working Group

Signature of Scientific Program Head: L.B. [illegible]

Date: 14/9/81

Abstract Summary:

We plan to quantitate local (saliva, duodenal fluid, lavage) as well as systemic (serum) immune response to acute cholera infection. During the first phase of disease the duodenal fluid will be collected by a nylon yarn string left in situ for a few hours and then pulled out. This fluid will be analysed for antibodies to cholera LPS and [illegible] the number of viable vibrio organisms, and amount of [illegible]. Lavage studies will be done later, the last one 4 weeks after [illegible] to the hospital.

By correlating our results with the clinical data, we will be able to confirm the theory that secretory antibodies are responsible for the "natural" termination of the disease. These data will also provide a basis for future evaluation of potential new cholera vaccines and their efficacy to stimulate local immunity.

8 Reviews:

a. Research Involving Human Subjects: _____

b. Research Review Committee: _____

c. Director: _____

d. BMRC: _____

e. Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: These studies can be seen as a logical extension of the protocol on "Local Immune Response in Cholera" (77-011) by Dr. D. A. Sack. The long range goal thus is the development of good enteric vaccines. We hope that further studies of the interaction between pathogenetic and local immunological mechanisms during an acute attack of cholera will provide a firm basis for evaluating new cholera vaccines.

2. Background:

2.1 Intestinal flora in cholera

The normal human small intestine has a sparse microbial flora. This relatively aseptic condition is maintained by physiologic and immunologic mechanism like such as barriers, propulsive motility and secretory antibodies. In the initiation of infection with V. cholerae these barriers must be overcome.

Once colonization is underway, the organisms grow to relatively large population densities; at least 10^5-10^6/ml in luminal fluid samples from naso-jejunal tubes (1). Presumably the number of vibrios is higher on the mucosal surface. Mucosal colonization seems to be necessary for disease to occur. Although large numbers of vibrios proliferate densely in the lumen could conceivably produce illness, there is no experimental evidence that this occurs.

Gorbach studying the background flora noted ... persistence ... coliforms in the jejunum in 8 of 10 patients.

Following clinical recovery (3-7 days in cholera) the cholera vib... are no longer found in the intestinal tract and their presence seem... to be correlated to the indigenous microflora (1). Already on day 2, the fecal flora showed signs of recovery: Bacteroides were more prevale... than vibrios (2).

Data on the bacteriology of the small bowel did not show any relation... to physiological or clinical parameters. Working with volunteers, Levine (3) estimated the mean incubation time for cholera to ... hours (range 15-110 hours). The volunteers were intubated 10 hours after challenge and those who developed diarrhoea were heavily colonized in their upper small intestine, but vibrios were cultured only during late incubation or early in the course of diarrhoea.

Enterotoxin

When actively growing, the organisms produce the enterotoxins which are the mediators of the disease process. Although a proximity of the growing bacteria to the epithelial cells obviously facilitates toxin delivery to the site of action, it is clear that toxin introduced intraluminally can also effectively penetrate those normal barr... the small bowel.

... of the toxin is complete within two min... of contact ... is a lag of up to three hours before biological effect... observed. It has been suggested that this lag is due to the time ... on

for active subunit A to become detached from the B subunit and to transverse the membrance to stimulate adenylate cyclase on the inner surface of the cell membrance. Cholera toxin has been measured in admission stools (5) and the concentrations varied greatly from < 1ng/ml to 150 ng/ml, and decreased with time.

In a trial of GM_1 ganglioside in patients with cholera, the effects of GM-1 ganglioside suggests that free toxin produced in the gut lumen effects fluid loss early in cholera, but that later in the course of disease toxin which is inaccessible to luminal binding agents is the major stimulus of purging.

The greater effect of GM-1 ganglioside early in the course of disease and in patients who had been ill for a shorter time might be explained by a lag time in the attachment of vibrio to intestinal mucosa.

2.3 Local antibodies

A limited number of studies have dealt with the problem of antitoxic and/or antibacterial immunity in the gut itself.

Waldman et al (6) reported that two out of 12 cholera patients had a rise in vibrocidal antibodies in their small-intestinal secretions. None had detectable agglutinating antibody.

In a study from Calcutta (7) 125 volunteers were immunized with various cholera vaccines, two doses 14 days apart. Local immunity was tested by vibrioagglutination test, vibrocidal antibody test,

involved in a challenge experiment, but that antitoxin prevents secretion

of fluid which may be required for optimal vibrio proliferation. According to this interpretation, it is only when vibrios reach the colon that they are killed by non-immune mechanisms.

Lavage studies in cholera patients (8) demonstrated that 19 of 20 lavage specimens contained measurable neturalizating antibodies, 18 of 20 had anti-CT IgA and 8/11 had anti-LPS IgA. The sensitive ELISA was used for the antibody assays.

The duodenal capsule

Fasi et al (9) described a new technique for sampling duodenal contents: a length of nylon yarn is coiled inside a weighted gelatin capsule. One end of the string protrudes through a hole in the capsule so when the capsule is swallowed the line is delivered to the stomach and then to the duodenum. When the string some hours later is pulled back, mucus-stained duodenal mucus adhering to it may be removed for examination. The mucus traps desquamated cells, eggs and larvae and the test thus has been used to detect parasites like Strongyloides stercoralis and Giardia lamblia (10,11) as well as salmonella organisms in typhoid carriers (12). Only persons with a very sensitive gag reflex are bothered by the presence of the thread in the throat. Removal of the line is mildly disagreeable, but it is accomplished rapidly. Secretory antibodies at the mucosal surface of the intestine are very important (13). The mucus layer covering the epithelium may constitute

antibodies within itself and hold them close to the gut surface.

The duodenal capsule, compared with incubation, thus has the practical advantage to be much more convenient to the patient and the theoretical advantage - not yet proven - to reflect the events within the mucus layer more than in the gut lumen itself.

SPECIFIC AIMS

Methods for assaying local immunity and toxin production have been developed at ICDDR,B and are now used in many protocols. The methods include a sensitive ganglioside-enzyme linked immunosorbent assay for the toxin levels and ELISA for cholera toxin-(CT) and LPS-antibodies. This gives us a unique opportunity to study patients with acute cholera and further document and quantitate the local immune response to cholera in Bengali adults. This would include determining the peak response, the duration between antigen exposure to peak response and the duration of measureable response.

Relating the immune response to the presence and disappearance of vibrios and enterotoxin may confirm the theory that secretory antibodies are responsible for the "natural" termination of the disease (16).

The correlation between local and systemic (serum) and local (faecal) immunity will also be determined.

OBSERVANCES: Since we are interested in the sequence of events in natural disease, the use of antibiotics would profoundly change the conditions and ruin the study. We believe that withholding antibiotics under these circumstances can be accepted. The antibiotic is generally used in order to shorten the diarrhoea and reduce the need for intravenous and oral fluids. In addition, use of an antibiotic will decrease the potential for contaminatin the environment (13). These patients will be optimally supervised regarding their hydration status and if ... stool cultures on day 2 still is positive for V. cholera, tetracycline will be administered to avoid spread in the community.

ETHODS OR PROCEDURE

Adult patients, male as well as female, presenting to ICDDR,B treatment centre with a history of acute watery diarrhoea (duration less than 12 hours). They should be moderate or severely dehydrated. No prior medication with antibiotics is allowed. If dark field positive they will be transferred to the study ward (baseline purging rate is not necessary). Informed written consent will be obtained. Rehydration with iv fluids will be given and iv fluids maintained for 72 hours. Fluid for oral rehydration will not be given, but a soft diet will be allowed. Antibiotics will be administered only when indicated by the clinical condition of the patients. Intake, output and weight will be recorded [illegible]

Patients will be hospitalized for 10 days during their acute illness and will be studied at ... hours following discharge.

A total number of 15 patients will be studied.

2. Collection of specimens

The clinical specimens in the table below will be obtained.

Day	Duodenal juice	Lavage	Saliva	Serum
1	X		X	X
3	X		X	X
5	X		X	X
9		X	X	X
17	X	X	X	X
28	X	X	X	X

Duodenal juice will be collected with the duodenal capsule described earlier. After at least two hours fasting the capsule is swallowed and the proximal end of the line is taped to the face and left in place for 2 hours during which time the patient may walk about, but not eat. Drinking is allowed - only water.

Then the line is retrieved from the mouth. The bile-stained and (alkaline) clinging to the distal 20 to 30 cm is then squeezed by squeezing the line between two fingers into a small container. Five drops are obtained.

This is diluted 1:50 in PBS in preweighed vials and one aliquot taken for viable count of vibrios.

rest is heated to 56°C for 15 minutes to inacti proteolytic
ves. The material is then aliquoted and frozen -70°C until
.

lavage specimens will be collected by letting the volunteers drink
isotonic salt solution until a watery diarrhoea ensues. The
nking is then continued until 1000 cc of watery stools has been
lected. This usually means that the patient has to drink 2-3
0 cc fluid. The passed liquid lavage stool is heated to 56°C for
minutes to inactivate proteolytic enzymes. Of this material 500 cc
sterilefiltered and concentrated to 10 ml by means of negative
ssure dialysis. The concentrated material is then aliquoted and
zen at -70°C. This procedure has been used in ICDDR,B since 1979.
ing tests are done before the lavage (day 0, 1 and 28).

stimulate the production of saliva the patients will chew on Parafi
a few minutes, 6-10 ml of saliva is collected, heat - inactivated
- after centrifugation - aliquoted and stored in -70°C until used.

serum will be obtained from a finger prick specimen of blood.
sposable needles and tubes will be used. 200 µl of blood will be
ted at 1:10 in PBS, serum separated and finally frozen in aliquots.

ory assays

collected will be tested as shown in the Table

	Duodenal fluid	Lavage	Saliva	Serum
Viable counts of Vibrio cholerae	X	X(?)		
Enterotoxin	X			
Antibody to				
CT-IgA	X	X	X	X
LPS-IgA	X	X	X	X
CT-IgG				X
LPS-IgG				X

Viable counts 0.1 ml from several 10^{-1} dilutions from intestinal samples diluted 1:50 in PBS and from lavage specimens will be streaked on to different media:Monsur's media for Vibrio and Gelatin - agar for enterobacteria in general.

If the Gas Liquid Cromatograph (GLC) is working at the time of the study an extended study of the gut flora will be done on the intestinal samples including several selective and non-selective media for aerobic and anaerobic culture. A separate protocol will be written on the subject.

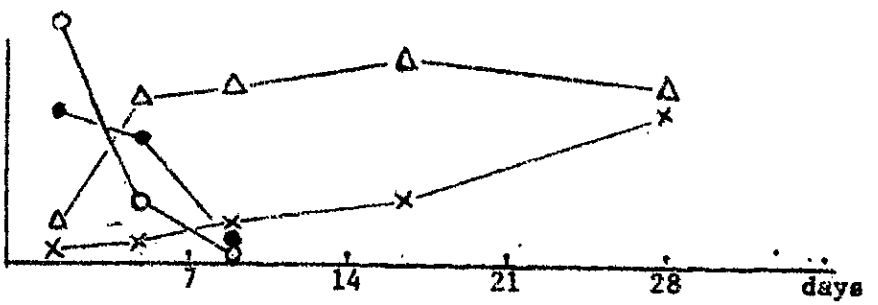
Enterotoxin will be measured with the GM-ELISA assay (Svennerholm A.M., Holmgren J. Identification of E. coli heat-labile enterotoxin by means of a ganglioside immunosorbent assay GM-1-ELISA. (Procedure Curr 1978; 1:19-27).

Antitoxic and antibacterial antibodies will be determined with the ELIS

4. Data analysis

The various data obtained will be analyzed and can be presented in the following way:

Specific aim 1 and 2



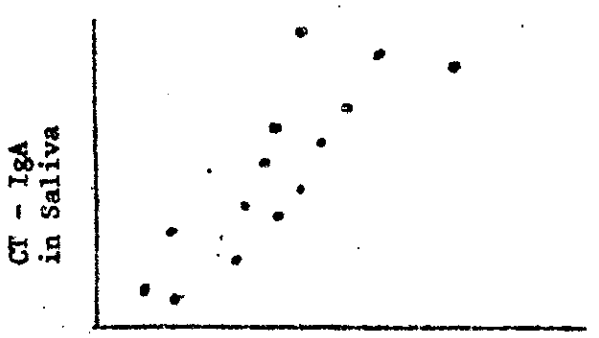
O—O Viable count of V. cholerae

●—● Cholera toxin

X—X antibodies to toxin

Δ—Δ antibodies to LPS

Specific aim 3



D. SIGNIFICANCE

The development of cholera and other enteric vaccines, which would stimulate local immunity, depends on the ability to quantitate the local immune response. This study is a continuation on Protocol 77-011 and will thus try to give further data on how local immunity to cholera develops and whether this immunity has a terminating effect on the actual illness or just gives protection to reinfection.

E. FACILITIES REQUIRED

Office and laboratory space is already existing within the Immunology and Microbiology Branch.

Hospital resources: Patients will remain in the hospital for 10 days. 15 patients x 10 days = 150 patient days. In addition patients will return for follow-up day 17 and day 28-30 follow up visits.

Logistic support: Follow-up visits will require pick-up of patients by transport in most cases.

20 patients x 2 pickups x 10 miles/pickup = 400 miles.

Specialized requirements - in some cases, in order to encourage cooperation from patients (esp. from mothers with other small children) we may have to provide special facilities or support. The follow-up outpatients visits will require the reimbursement to the subjects for expenses of travelling to the hospital and loss of daily wages (Tk.25/day). As they have to stay in hospital perhaps one or two extra days in order to do the lavage on day 9, Tk.50 will be given as a cover of lost wages.

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ABSTRACT SUMMARY

1. Only adult patients with cholera will be included in this study.

2. The risks from this study are minimal and consist of the repeated discomfort of swallowing the capsule plus string for acquiring duodenal fluid. All patients will be treated with appropriate fluid therapy.

3. The dehydration will be treated optimally. If the patient in spite of that is deteriorating tetracycline will be given and the patient excluded from the study. Antibiotics will also be administered one day before discharge, if the patient at that time is still culture positive for V. cholerae.

4. All patients will be identified by patient number and all records will be kept locked in the investigator's office.

5. All subjects will sign a consent form (see enclosed form).

6. N.A.

7. The individual will gain no personal medical benefit other than treatment for cholera which he/she would receive regardless of the study. She will however, receive payment for the lavage days (Tk. 25/day). Society will benefit if this study can give more information on local immunity to cholera and thus provide a basis for evaluation of potential new vaccines.

8. Blood, saliva, stool and duodenal secretion will be collected.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	Time & Effort	Annual salary	Taka	Dollar
Leif Gothefors	Investigator	3 months 50%	48,660	-	6,080
David Sack	Co-Investigator	10%	50,400	-	5,040
A. Khanam	Co-Investigator	15% 5 months	57,600	3,600	-
G.H. Rabbani	Co-Investigator	15% 3 months	58,160	2,200	-
Jan Holmgren		10%	-	-	No cost
A.M.Svennerholm		20%	-	-	No cost
Field Assistant		5 months		10,000	-
Lab. staff(Immuno)	4 for 3 for	3 months 4 months		48,000	-
Nurse study ward	2 for	3 months		12,000	-
			Sub total :	75,800	11,120

2. SUPPLIES AND MATERIALS

Plastic, glassware (plates, pipette tips, tubes), dialysis bags	-	3,000
Reagents (antisera, conjugates, enzymes)	-	2,000
Misc. clinical supplies	-	1,000
Sub total	0	6,000

3. EQUIPMENT

Nil

4. HOSPITALIZATION COSTS

180 patient days	27,000	-
Sub total	27,000	0

5. OUTPATIENT CARE

0

6. LOCAL TRANSPORT

Mileage 400 miles

Sub Total : 800

7. TRAVEL

Local - None
International - one roundtrip for foreign Sovereignsor

Sub Total : 0

8. TRANSPORTATION OF THINGS

Sub Total : 0

9. RENT, COMMUNICATION, UTENSILS

Postage, cables

Sub Total : 0

10. PRINTING, REPRODUCTION

Forms- mail, xerox, publication costs

Sub Total : 1,000

11. OTHER CONTRACTUAL SERVICES

Tuition fees

Sub Total : 1,000

12. [illegible]

0

TOTAL :

Grand Total :

(U.S.$ 1.00 = Dr. 16.00)

B. BUDGET SUMMARY

		Dollar
1.	Personnel Services	15855
2.	Supplies and Materials	6000
3.	Equipment	-
4.	Hospitalization costs	1690
5.	Outpatient Care	-
6.	ICDDR,B Transport	50
7.	Travel	3000
8.	Transport of Things	1000
9.	Rent, Communication	100
10.	Printing	460
11.	Contractual Service	95
12.	Construction	-
	Total US$	28,250.00

CONSENT FORM

LOCAL IMMUNE RESPONSE IN CHOLERA

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out research to better understand how to protect people from cholera and other diarrhoeal diseases. We would like you to participate in a study to determine the immune (protective) response which occures when a person develops cholera. We hope that the information we gain will be helpful in developing a new cholera vaccine which will be much more effective in preventing cholera. If you agree to participate in this study, you can expect the following:

1. You will need to stay in the hospital for 10 days; also you will need to return to the hospital for two more days. We will pay you for the cost of transportation plus 25 Taka for each of these two days and 50 Taka for the extra time you have to spend at the hospital.

2. During your hospitalization we will collect blood and saliva 4 times. Also on 4 occasions we will pass a thin string into your intestine to collect a specimen of intestinal juice.

3. After your discharge from the hospital we will collect blood, saliva and intestinal juice specimens at two occasions.

   We will also have you do the intestinal lavage procedure 3 times. This is a procedure in which you will drink a large volume (up to 5 liters) of salty water and this will cause a temporary diarrhoea. The diarrhoea stops shortly after you stop drinking the salty water. During the lavage you will have a full feeling in the abdomen, you will gain 1-3 kg in weight but you will not have pain or any serious side-effects.

4. None of the tests are harmful to your health. Drawing blood and passing the intestinal string are somewhat uncomfortable; they do not have any serious side effects.

../2

5. Your medical records will be kept confidential.

6. You do not have to participate in the study. Your decision concerning the study will not effect your medical treatment while in the hospital. If you do enter the study, you are free to leave the study at any time without jeopardizing your medical care. We will answer any questions you have concerning the study.

If you agree to participate in this study, please sign your name here.

_____

_____ Date

_____

Investigator's signature

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র
বাংলাদেশ

সম্মতি পত্র

এই আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্রটি কলেরা এবং অন্যান্য উদরাময়ের রোগ থেকে মানুষকে মুক্ত করার জন্য একটি ব্যাপক অনুসন্ধান কার্য চালিয়ে যাচ্ছে। আমরা (অনুসন্ধানকারী) চাই কলেরা বা উদরাময় দ্বারা আক্রান্ত হবার পর আপনার শরীরে কতটুকু রোগ প্রতিরোধ করার শক্তি জন্মেছে তা পরিমাপ করার জন্য আপনি আমাদের এই গবেষনায় অংশ গ্রহন করুন। এরদ্বারা আমরা যে সব খবর সংগ্রহ করতে পারবো তা কলেরা প্রতিরোধ টিকা আবিস্কারে খুবই সহায়ক হবে।

আপনি যদি এই গবেষণায় অংশগ্রহণ করতে ইচ্ছুক হন তবে নিম্নলিখিত পদ্ধতিগুলো অনুসরণ করুন :

১। আপনাকে ১০ দিন হাসপাতালে থাকতে হবে - এছাড়াও পরে দুদিন আপনাকে এখানে আবার আসতে হবে। এই দুদিনের জন্য আমরা আপনাকে যাতায়ত খরচ ও ২৫ টাকা করে হাত খরচ দেবো এছাড়া আরো বেশী সময় থাকতে হলে আরো ৫০ টাকা আলাদা করে দেবো।

২। হাসপাতালে থাকাকালীন আমরা ৪ বার আপনার রক্ত ও থুথু সংগ্রহ করবো। এছাড়া আরো ৪ বার একটি সরু নল আপনার পাকস্থলীতে প্রবেশ করিয়ে কিছু পেটের ভিতরকার রস সংগ্রহ করবো।

৩। আপনার হাসপাতাল ত্যাগ করবার কিছুদিন পর আবার দুইবার আপনার রক্ত, থুথু এবং পাকস্থলীর রস পরীক্ষা করবো।

এছাড়া ৩বার আপনার তরল পায়খানা পরীক্ষা করবো। তখন আপনাকে ৩ বার লবন পানি খেয়ে সাময়িকভাবে তরল পায়খানা করতে হবে। এতে আপনার কোন প্রকার বাধা বা বিরূপ প্রতিক্রিয়া হবে না। তবে পেটটা একটু ভার ভার বোধ করতে পারেন। লবন পানি খাওয়া বন্ধ করার পর কিছুক্ষনের মধ্যে তরল পায়খানা বন্ধ হয়ে যাবে।

৪। এই সব পরীক্ষা যেমন রক্ত নেওয়া, পেটের ভিতরে নল ঢুকানো ইত্যাদি কোনটাই আপনার জন্য কষ্টকর বড় জোর সামান্য অস্বস্তিকর লাগা ছাড়া। এগুলোর কোন বিরূপ প্রতিক্রিয়াও নেই।

৫। আপনার চিকিৎসা সম্পর্কীয় সকল তথ্যাদি গোপন রাখা হবে।

৬। আপনি এই গবেষণায় অংশগ্রহণ না করলেও আপনার চিকিৎসা করা হবে। আপনি ইচ্ছে করলে যে কোন সময় এই গবেষণা পরিত্যাগ করতে পারেন। তাতে চিকিৎসার কোন তারতম্য ঘটবেনা।

আপনি এ গবেষণায় অংশগ্রহণে রাজী থাকলে এখানে আপনার স্বাক্ষর দিন-

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স্বাক্ষর / টিপসহি

তারিখ----------

অনুসন্ধানকারীর স্বাক্ষর