REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS
CRL

Date 15 Jan 1978

Principal Investigator David A. Sack

Trainee investigator(if any) _____

Application No 78-005

Supporting Agency(if Non-CRL) _____

Title of study Local Immune Response to the Field Trial Cholera Vaccine

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 possibly 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 N/A
   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
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 Board for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

78-005
Rec'd 1/1/78

SECTION I - PROJECT DESCRIPTION

1) Title: Local Immune Response to the Field Trial Cholera Vaccines.

2) Principle Investigator: David A. Sack, M.D.

3) Starting Date: January, 1978.

4) Completion Date: January, 1979.

5) Total Direct Cost: 28,477

6) Abstract Summary: This study is planned to determine the effect of the field trial cholera vaccines on the local antitoxic immune system. The study will be divided into two sections. Part "A" will be an assessment of antitoxin titers in breast milk samples of lactating women receiving the vaccines. Part "B" will be an assessment of antitoxic immunity in blood, saliva and intestinal washings from a group of healthy volunteers in Dacca who receive the vaccine in an identical manner. It is hoped that these studies will help explain the results observed in the vaccine field trial and will help in understanding the basic mechanism of the local immune system in relation to cholera.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

A. INTRODUCITON

1. Objective: The objective of this protocol is an understanding of the effect of the systemically administered cholera vaccine on the local immune system of the gut.

2. Background: During 1978 a field trial of two new cholera vaccines will be carried out in the Matlab area, Bangladesh. The vaccines in the trial include a purified toxoid vaccine, a whole cell vaccine and a combination of the toxoid and whole cell. These vaccines will all contain an aluminum adjuvant. Tetanus toxoid will serve as the control in a four cell double blind trial. This field trial is being carried out to evaluate several questions:

   (1) Is cholera toxoid an effective means of inducing protection from cholera?

   (2) Does the addition of aluminum adjuvant enhance and prolong the protection from whole cell vaccine?

   (3) Is there evidence of synergistic protection in patients receiving the combination toxoid plus whole cell vaccine?

The protection (or lack of protection) that may be seen from these vaccines will certainly be the result of a number of immunologic events induced in the systemic and local immune systems of the recipients and it is the purpose of this protocol to describe one of these mechanisms, namely, the events occurring in the local immune system of the gut.

Cholera is a disease, the pathophysiology of which is totally localized to the lumen and surface of the small intestine. Neither the V. cholerae organisms nor its toxin invade the mucosa of gut; rather, the entire process occurs locally. It follows then that protection must also be a local process, and if the cholera vaccine is to be protective, it must either inhibit the colonization of the vibrio in the intestine or neutralize the toxin before it attaches to the $GM_1$ receptor. This "local" protection does not necessarily mean IgA antibody since IgG in very high titers in the blood can diffuse into the gut and protect against disease, and protection may even involve non-immunoglobulin protection. The most important immunoglobulin of local surfaces is IgA; however, and it therefore the focus of this protocol.

The relationship between the local IgA system and the systemic immune system is complex. Under certain conditions antigenic stimulation of one system leads to a response in the other and under other

circumstances a relative tolerance is induced. Trapping and neutralization of antigen, modification by T cells and probably other factors influence the stimulation or tolerance induced.

In the situation of the field trial, one would assume that the recipients had previously been "primed" locally to cholera antigens. That is, persons living in a cholera endemic area of rural Bangladesh, would have a high probability of having had either a clinical or subclinical episode of cholera in the past, perhaps on repeated occasions. Therefore the local immune system of the gut would have previously been exposed to both bacterial and toxin antigens and would have initiated an immune response to these antigens. During that previous exposure(s) a systemic immune response would also have likely occurred so that the recipient of the vaccine, even before the vaccine, has both a primed local and systemic immune system.

When the cholera vaccine is given intramuscularly it will certainly stimulate a systemic IgG (and perhaps a more transient IgM) response. This IgG response may induce a high titer of antibody in the blood so that sufficient antibody will diffuse into the gut to aid in protection. It should be clear; however, that the circulating IgG is not per se protective but is rather only an indication of the immune response. The effect of the cholera vaccine on the local IgA system of the gut is not known. One hypothesis is that the first dose of vaccine will stimulate the local system but the second dose will not. This would be explained by a small level of antigen which might reach Peyer's Patches via the blood stream with the first dose; however, with the second dose, the higher levels of systemic antibody would absorb and "protect" the local immune from the second antigenic challenge. The knowledge of the stimulation of the local immune system is limited, however so that we cannot predict the actual local events.

Documentation of a local gut immune response has been difficult. The most direct means is through sampling intestinal fluid via intestinal intubation, and measuring the sample for antibody content. We propose to alter the procedure so that we will sample intestinal contents without intubation using an intestinal lavage. Using this method we will obtain a sample from the entire intestine to assay for antibody content.

Other methods of assessing local immunity is through sampling the milk of lactating women as a proxy measure of gut fluid. The immune system of the gut is closely linked with the breast and there is evidence that the IgA antibodies in normal breast milk are primarily determined by local immune events in the gut.

One other proxy measurement of gut immunity may be the assay of parotid fluid for IgA antibody. The relationship immunologically of the parotid gland with the gut is not known; however, parotid fluid is easily obtained, and measurement of antibody titer in this fluid may represent the assessment of the immune response in a local system not previously primed by cholera antigens.

The specific antibodies to be measured include antitoxic and neutralization. The antibody assays would use the ELISA and the Y, adrenal and mouse lymphome neutralization assays. Antibacterial antibodies will be measured by the Elisa LPS assay.

3. Rationale: During the 1978 vaccine field trial we plan to assess one important mechanism of protection, the local immune system and the effect of the vaccines on this system. The will help to interpret the clinical results of the trial and will enhance the basic understanding of the local immune system as it applies to cholera.

B. SPECIFIC AIMS

1. Describe the local antitoxic and antibacterial immune response which occurs with the field trial vaccines.

2. Compare the immune response of the first dose with the immune response of the second dose of vaccine.

3. Compare the local immune response in patients who receive the single vaccine with those who recieve the combination (using the tetanus group as a control).

C. METHODS OF PROCEDURE

The subjects to be studies are in two groups. Each will be described.

Group A: Lactating women, whose youngest child is one year of age or less, and who will be receiving one of the cholera vaccines will be asked to contribute milk samples during the pretest period of the trial. Oral consent will be obtained and milk will be obtained shortly before the first dose of vaccine, on day five and day 14 after the dose. This same schedule will be repeated with the second dose of vaccine so that each woman would submit six samples. Fifteen women will be required from each of the four cells of the study so that at least ten complete collections from each cell will be available for analysis. Five to ten ml of milk will be obtained with each collection. No other specimens will be required from women other than those finger stick blood specimens required for the vaccine pretrial (see protocol by M. Merson).

group B: Volunteers from Dacca will be recruited for special studies of local immunity. Approximately 24 male Bangladeshi persons aged 20-40 years will be given two doses of one of the cholera vaccines (six volunteers per cell) in a manner identical with the schedule in the vaccine field trial. Special studies on these patients will include: 1) venapuncture, 2) parotid duct fluid collection, and 3) intestinal lavage. The procedures will be carried out on the day of first injection (day 0), on day five and 14 after injection for each of the two doses. Ten ml of blood will be obtained with each venapuncture. Parotid fluid will be collected using a small cupule over Stenson's

duct. This is a completely non-invasive procedure to collect parotid fluid. Intestinal lavage will be performed by using the technique as described for barium enema preparation and collecting the diarrheal stool for analysis.

Handling of specimens: The serum will be alequotted in one ml alequots and frozen, except for two alequots which will be lyophilized. The parotid fluid will be inactivated at 56° C for 15 minutes, then alequotted into one ml alequots with two alequots being lyphilized and the remainder frozen. The diarrheal stool will be inactivated at 56° C for 15 minutes immediately after it is passed. It will then be filtered through gauze, contrifuged at 10,000 x G; the supernate will be filtered through 0.45 microfilter and the filtrate will be concentrated by negative pressure dialysis followed by lyophilization. Milk specimens collected in the field will be kept in a cold box until the return to Matlab Hospital where they will be inactivated at 56° C for 15 minutes. They will then be kept cold until their delivery to Dacca within two days of collection. In Dacca they will be centrifuged at 15,000 x G (to defat) and the whey will be alequotted into one ml alequots except for two alequots which will be lyophilized.

All of these specimens will be analyzed in the following assays. One, neutralization assay using the $Y_1$ adrenal cell assay and the mouse lymphoma assay. The microtiter adrenal cell assay is established at the CRL and is performed by mixing a standard quantity of cholera toxin with serial dilutions of test sample and then transfering this mixture to the adrenal cell plate. The last dilution which completely neutralizes the toxin (as evidenced by inhibition of rounding) is the end point and is compared to a standard antibody. The mouse lymphoma assay is also performed in a microtiter plate and is a similar assay except that the neutralization of toxin evidenced by a pH change in media caused by cell growth. The mouse lymphoma assay is currently being done in the laboratory of Dr. N. Pierce.

The ELISA assay for CT is now established at CRL and the assay for IgA and IgG antitoxin will be shortly. This is also done in microtiter and is an Ig class apecific binding assay which is similar to a solid phase radio immune assay except that an enzyme lable, rather than a radio labelled antibody, is used. The ELISA assay for L.P.S. antibody has been described and will either be set up at CRL or done in the laboratory of Dr. Jan Holmgren.

Data analysis will be by comparing mean geometric titers of antibody (in units/mg specific Ig) between the time intervals listed using a paired T test and in comparing significant (i.e. four fold) rises in titer in the fluids tested which occurs in response to the vaccines using a Fisher's exact test.

D. SIGNIFICANCE

This study will help to assess one of the mechanisms of protection which the cholera vaccine will produce. That is, it will help explain the results observed in the field. It will also add to a basic understanding of the local immune system at least as it applies to cholera.

E. FACILITIES REQUIRED

1. Office Space is already provided.

2. Laboratory Space is already provided.

3. Hospital Resources: Volunteers will be studied in the hospital. A total of 144 patient days will be needed.

4. Animal Resources: None.

5. Logistic Support: The vaccine trial field staff with Dr. Merson supervising will collect breast milk samples in the field. The milk samples will require approximately one hour of speedboat time on each of six days. Transport of milk samples to Dacca will be by regular transport.

6. Major Items of Equipment: NIH type Elisa reader.*

7. Specialized Requirements: Since this study will involve volunteers we will have to provide special services during their stay at CRL including transport allowance, payment for volunteering (Tk. 50 per day), and a pleasant room for the intestinal lavage procedure.

F. COLLABORATIVE ARRANGEMENTS

Dr. Nate Pierce will perform the lymphoma cell neutralization assay. Dr. Jan Holmgren will assist in setting up the LPS ELISA assay at CRL.

* The Elisa reader will be used in all the Elisa assays for antibody. The only reader available commercially is not suitable for CRL. We would plan then to work through NIH which has designed a suitable reader who will contact with a firm to make such as an instrument. It is estimated to cost approximately $5,000.

## REFERENCES


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Andre, C., Heremans, J.F., Vaerman, J.P., & Cambiaso, C.L. A Mechanism for the Induction of Immunological Tolerance by Antigen Feeding: Antigen-Antibody Complexes. J. Exper. Med. 142: 1509-1519, 1975.

Freter, R. Detection of Coproantibody and Its Formation After Parenteral and Oral Immunization of Human Volunteers. J. Infect. Dis. 111: 37-48, 1962.

Northrup, R.S. & Hossain, S.A. Immunoglobulins and Antibody Activity in the Intestine and Serum in Cholera. II. Measurement of Antibody Activity in Jejunal Aspirates and Sera of Cholera Patients by Radioimmunodiffusion. J. Infect. Dis. 121 (Suppl.): S142-S147, 1970.

Pierce, N.F. Intestinal immunization with soluble bacterial antigens: the example of cholera toxoid. Acute Diarrhoea in Childhood Ciba Foundation Symposium 42, pub. Elsevier/Excerpta Medica/North-Holland, July 1976, pgs. 129-147.

Thomas, H.C. & Parrott, D.M.V. The Induction of Tolerance to a Soluble Protein Antigen by Oral Administration. Immunology 27: 631-639, 1974.

Waldman, R.H., Bencic, Z., Sinha, R., Deb, B.C., Sakazaki, R., Tamura, K., Mukerjee, S., & Ganguly R. Cholera Immunology. II. Serum and Intestinal Secretion Antibody Response after Naturally Occurring Cholera. J. Infect. Dis. 126(4): 401-407, 1972.

Ujiye, A. & Kobari, K. Protective Effect on Infections with Vibrio cholerae in Suckling Mice Caused by the Passive Immunization with Milk of Immune Mothers. J. Infect. Dis. 121(Suppl.): S50-S55, 1970.

SECTION III - BUDGET

## A. DETAILED BUDGET

### 1. PERSONNEL SERVICES

| | Name | Position | Percent of effort or number of days | Annual Salary | Project Requirements TAKA | Project Requirements DOLLARS |
|---|---|---|---|---|---|---|
| 1. | Dr. David Sack | Investigator | 30 | $ 34,750 | | 17,375 |
| 2. | Study Physicians (to be named) | Physician | 20 | Tk.28,000 | 5,600 | |
| 3. | Mr. Neogi | Technician | 40 | Tk.25,000 | 10,000 | |
| 4. | 2 Technicians (to be named) | Technician | 10 | Tk.20,000 | 4,000 | |
| 5. | Dr. Michael Merson | Investigator | | | | |
| | | | | Sub Total: | 19,600 | 17,375 |

### 2. SUPPLIES AND MATERIALS

| Item | Unit Cost | Amount Required | Project Requirements TAKA | Project Requirements DOLLARS |
|---|---|---|---|---|
| Glassware, plastics, media | | | 5,000 | 2,000 |
| | | Sub Total: | 5,000 | 2,000 |

### 3. EQUIPMENT

| | |
|---|---|
| NIH type Elisa reader | 5,000 |

### 4. PATIENT HOSPITALIZATION

| | | |
|---|---|---|
| Number of patient days - 144 (times Tk.135 per day) | 19,440 | |
| Sub Total: | 19,440 | |

### 5. OUTPATIENT CARE

None

| | Project Requirements | |
|---|---|---|
| | TAKA | DOLLARS |
| 6. CRL TRANSPORT | | |
| Hours-Water Transport: 6 | 600 | |
| Sub Total: | 600 | |
| 7. TRAVEL AND TRANSPORTATION OF PERSONS | | |
| None | | |
| 8. TRANSPORTATION OF THINGS | | |
| | | 500 |
| Sub Total: | | 500 |
| 9. RENT, COMMUNICATIONS & UTILITIES | | |
| | | 20 |
| Sub Total: | | 20 |
| 10. PRINTING AND REPRODUCTION | | |
| | 1000 | |
| Sub Total: | 1000 | |
| 11. OTHER CONTRACTUAL SERVICES | | |
| Volunteer payments (24 volunteers) | 7200 | |
| Sub Total: | 7200 | |
| 12. CONSTRUCTION, RENOVATION, ALTERATIONS | | |
| None | | |

## B. BUDGET SUMMARY

| Category | Project Requirements TAKA | DOLLARS |
|---|---|---|
| 1. Personnel | 19,600 | 17,375 |
| 2. Supplies | 5,000 | 2,000 |
| 3. Equipment | | 5,000 |
| 4. Hospitalization | 19,440 | |
| 5. Outpatients | | |
| 6. CRL Transport | 600 | |
| 7. Travel Persons | | |
| 8. Transportation Things | | 500 |
| 9. Rent/Communication | | 20 |
| 10. Printing/Reproduction | 1,000 | |
| 11. Contractual Service | 7,200 | |
| 12. Construction | | |
| Total: | 52,840 | 24,895 |
| Total $ | 28,417 | |

Conversion Rate $ 1.00 : Tk. 15/

## ABSTRACT SUMMARY

1. The study population will include two groups of people. The first group will be lactating women who are receiving one of the cholera vaccines in the 1978 field trial. The second group will be young adult male healthy volunteers (age 20-40) recruited from the Dacca area.

2. There will be no risk to the collection of breast milk samples from the lactating women. In the second group (the volunteers), venapuncture blood (5ml), and parotid fluid will be obtained and the intestinal lavage procedure will be done six times. The venapuncture is uncomfortable but does not have a risk. The collection of parotid fluid is completely painless and has no risk. The intestinal lavage procedure consists of having the volunteer drink isosmotic balanced salt solution at the rate of 250ml every 10 minutes for 4-5 hours, which is an effective purgative. Within approximately 2 hours he will have a bowel movement and within 3 hours he will have diarrhea. When the drinking is discontinued the purging also ceases. Side effects from this procedure include a temporary weight gain of 1-2kg due to absorbed water and salt and a full feeling in the abdomen. There is no cramps or tenismus, the diarrhea is painless. Because of the salt load, persons with any heart disease, renal disease or hyper-tension will not be included. This procedure is used to evacuate the bowel of feces in preparation for a barium enema.

3. Since the intestinal lavage has a large salt and water load, persons with heart disease, kidney disease or hyper-tension will not be accepted as volunteers.

4. The lactating mothers who will be donating milk samples will be identified by code number only, and no name or address will be recorded. The names of the volunteers will be listed, however, only code numbers will be used in labelling, assays, and analysis. The sheet linking the volunteers with the code number will be kept in the investigator's locked office.

5. Verbal consent will be obtained from the mothers donating breast milk. Written consent will be obtained from volunteers who participate in the second section.

6. No interview.

7. The individuals receiving the vaccine will likely benefit from the vaccine but will not benefit from this evaluation of the vaccine. Society will benefit if these studies lead to an effective cholera vaccine. The risks are minimal.

8. The study will require the use of milk, blood, parotid and diarrheal fluid.

# PERMISSION FORM

Verbal consent will be obtained from mothers submitting breast milk samples. The following information will be read to them in Bengali.

The CRL is doing some special studies in relation to the vaccine field trial. These studies are intended to help assess the effect of the vaccine on the immune (protective) system of the body. We would like you to participate in this special study by allowing us to have small samples of breast milk on 6 occasions during the vaccine field trial. The samples of milk will be used to help determine the effectiveness of the vaccine.

You do not have participate in this breast milk collection and your decision to participate will not affect your treatment at the CRL hospital. You may withdraw from the study if you so desire at any time.

Your records will be kept confidential.

# PERMISSION FORM

The Cholera Research Hospital is doing some special studies to determine the way in which cholera vaccine helps to protect people from cholera. The study is designed to study the effect of the vaccine on the immune (protective) system of the intestine. We would like you to participate in this study if you are willing. If you decide to volunteer, you can expect the following:

1. You will receive two doses of a vaccine used in the 1978 cholera vaccine field trial. The vaccine which you will receive will be one of the following: a cholera toxoid vaccine, a whole cell vaccine, a combination of whole cell and toxoid or tetanus toxoid. At the time you receive the injection you will not know which of the vaccines you have received; however, we will tell you after the studies are complete. The vaccine will be given 6 weeks apart.

2. On the day you receive the first dose you would come to the clinical research ward of the CRL where we will obtain samples of blood, saliva and stool. To obtain stool you would have to drink a large amount of salty water (about a liter) over 4 to 5 hours. When you drink this water you will develop some watery stools which we will collect. When you stop drinking, the watery stool will also stop. This procedure, called intestinal lavage, will make your stomach feel full but it is neither painful nor dangerous.

3. You would need to return to CRL to repeat these studies 5 days after the vaccine and 14 days after the vaccine, and again repeat them the day of the second dose of vaccine and 5 and 14 days after the second dose.

4. You do not have to participate in this study and you may withdraw from the study at any time.

5. Your records will be kept strictly confidential.

6. You will be paid Tk50 for each completed day of study. This will be paid as Tk 50 at the end of the six sessions.

7. You may ask questions concerning the study at any time.

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

_____

_____

Date

## " সম্মতি পত্র"

যে সব মায়েদের বুকের দুধ নেওয়া হবে তাদের কাছ থেকে মৌখিক সম্মতি গ্রহন করা হবে । তাদেরকে নিম্ন বর্নিত বিবরন-গুলি পরিস্কার বাংলায় পড়ে শোনান হবে ।

কলেরা গবেষনাগার কয়েকটি বিশেষ ধরনের গবেষনা চালিয়ে যাচ্ছে যাতে করে পল্লী এলাকার জন্য একটি প্রতিশেধক উষধ বের করা যায় । এই গবেষনার দ্বারা মানব দেহের রোগ প্রতিরোধ ক্ষমতার উপর টিকা বা প্রতিশেধক উষধের কার্য্যকারিতা কিরুপ হবে তা জানা অতি সহজ হয়ে উঠবে । আমরা আশা করব যে আপনি ও এই গবেষনায় অংশ গ্রহন করবেন । পল্লী এলাকায় এই প্রতিশেধক টিকার কার্য্যকারিতা পরীক্ষা চলাকালীন আপনার কাছ থেকে ছয় বার বুকের দুধ সামান্য পরিমানে গ্রহন করা হবে । এটা থেকে প্রতিশেধক টিকার রোগ প্রতিরোধ ক্ষমতা সম্পর্কে জ্ঞান অর্জন করা যাবে ।

আপনি ইচ্ছা করলে বুকের দুধ না দিতে পারেন । এবং গবেষনায় অংশ নাও নিতে পারেন । তাতে এই হাসপাতালে আপনার স্বাভাবিক চিকিৎসার কোনরুপ ত্রুটি হবে না । গবেষনায় ভর্তি হবার পর ও যে কোন সময় গবেষনা পরিত্যাগ করে চলে যাবার অধিকার আপনার থাকবে ।

আপনার ডাক্তারী পরীক্ষার ফলাফল সম্পূর্ন গোপন রাখা হবে ।

"সম্মতি পত্র"

কলেরা রোগের প্রতিশেধক টিকার কার্য্য কারিতা সম্পর্কে কলেরা গবেষনাগার বর্তমানে কয়েকটি বিশেষ ধরনের গবেষনা চালিয়ে যাচ্ছে । মানব দেহের পাচক নাড়ীর যে রোগ প্রতি রোধ ক্ষমতা রয়েছে তার উপর এই ধরনের কলেরার টিকা বা ইনজেকশনের কি প্রভাব হতে পারে সেটা জানার জন্য এই গবেষনার পরিকল্পনা করা হয়েছে । আপনি ইচ্ছা করলে এই গবেষনায় অংশ গ্রহন করতে পারেন । যদি আপনী অংশ গ্রহনে সম্মত থাকেন তবে নিম্নবর্নিত বিষয়গুলি আপনার উপর প্রয়োগ করা হবে ।

১) ১৯৭৮ সালের কলেরার ইনজেকশন( Cholera Vaccine )ফিল্ড ট্রায়াল গবেষনায় আপনাকে দুই বার কলেরার ইনজেকশান দেয়া হবে । প্রধানত কয়েক ধরনের ইনজেকশান ব্যবহার করা হবে । একটি হল কলেরা টক্সোয়েড( Toxoid ), আর একটি হল জীবানুর জীবদেহ থেকে প্রস্তুত ইনজেকশান, আর অন্যটি হল কলেরা টক্সোয়েড( Toxoid ) ও জীবদেহ ইনজেকশানের মিশ্রনে প্রস্তুত ইনজেকশান । অথবা জীবদেহ( Vaccine ) এবং ধনুষ্টঙ্কার রোগের টক্সোয়েড ( Tetenus Toxoid ) এর সংমিশ্রন । প্রথম বার ইনজেকশান প্রয়োগের সময় কোন ধরনের ঔষধ ব্যাবহার করা হয়েছে- তা আপনাকে জানতে দেয়া হবেনা । অবশ্য পরিকল্পনা সমাপ্তির পর তা জানিয়ে দেয়া হবে । ইনজেকশান গুলী ছয় সপ্তাহ পর পর প্রয়োগ করা হবে ।

(২) প্রথম দিন ইনজেকশান নেবার জন্য আপনাকে কলেরা হাসপাতালে আসতে হবে । এই দিন পরীক্ষার জন্য আপনার নিকট থেকে সামান্য পরিমান রক্ত , থুথু ও মল রাখা হবে । পায়খানা পরীক্ষার জন্য আপনাকে আধাসের সামান্য লবনযুক্ত পানি পান করান হবে । চার থেকে পাঁচ ঘন্টার মধ্যে এই পানি পান করতে হবে । এই পানি পান করার পর আপনার সামান্য পাতলা পায়খানা হবে এবং সেটা পরীক্ষার জন্য আমরা সংগ্রহ করব । পানি পান করা শেষ হলে পাতলা পায়খানাও বন্ধ হয়ে যাবে । এই ধরনের পরীক্ষার সময় আপনার পেট সামান্য পরিমানে ফুলে উঠতে পারে । তবে এটা কোন প্রকার বেদনা দায়ক নয় এবং এ থেকে কোন ক্ষতির সম্ভবনা নেই ।

(৩) প্রথম ইনজেকশানের দিন থেকে পাঁচ দিন পর একবার এবং ১৪ দিন পর আর একবার আপনাকে হাসপাতালে আসতে হবে । এবং এই দুই বারই আপনাকে অনুরুধ লবন পানি খাওয়ানো পরীক্ষা করতে হবে । একই ভাবে দ্বিতীয় ইনজেকশানের ৫ দিন ও ১৪ দিন পর আপনাকে আর ও দুইবার হাসপাতালে আসতে হবে এবং উক্ত পরীক্ষার জন্য পানি পান করতে হবে ।

(৪) আপনী ইচ্ছা করলে গবেষনায় ভর্তি নাও হতে পারেন অথবা গবেষনায় অংশ গ্রহনের পর চলেও যেতে পারেন ।

(৫) আপনার ডাক্তারী পরীক্ষার কাগজ পত্র সম্পূর্নভাবে গোপন রাখা হবে ।

(৬) প্রতিদিনের পরীক্ষার জন্য আপনাকে টাকা হিসাবে দেয়া হবে । ছয়বার পরীক্ষা সমাপ্ত হলে সব টাকা আপনাকে বুঝিয়ে দেয়া হবে ।

(৭) আপনি গবেষনার ব্যাপারে যে কোন প্রশ্নাদি করতে পারবেন ।

সব কিছু অবগত হবার পর যদি আপনি গবেষনায় অংশ গ্রহন করতে রাজী থাকেন তবেনীচে আপনার নাম লিখুন । নামঃ----------

তাং----------