Date 19/7/84

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator DR. IHAN CIZNAR Trainee Investigator (if any) 2

Application No. 84-033 Supporting Agency (if Non-ICDDR,B)

Title of Study IMMUNOCHEMICAL ANALYSIS OF V. CHOLERAE ANTIGENS WITH EMPHASIS ON PHENOTYPIC VARIATIONS IN CARBOHYDRATE ANTIGENS; IMPLICATION FOR VACCINE DEVELOPMENT

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)
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
   NA Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

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

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

Ihan Ciznar
Principal Investigator

Trainee

84-033
19/7/84

SECTION I - RESEARCH PROTOCOL

1. TITLE : Immunochemical analysis of V. cholerae antigens with emphasis on phenotypic variations in carbohydrate antigenic determinants; implication for vaccine development

2. PRINCIPAL INVESTIGATOR : Dr. Ivan Ciznar

CO-INVESTIGATORS : Dr. Asma Khanam
Dr. Ansaruddin Ahmed
Dr. D. Sack

3. STARTING DATE : September 01, 1984

4. COMPLETION DATE : August 31, 1985

5. TOTAL DIRECT COST : US$ 62,568

6. SCIENTIFIC PROGRAM HEAD : T. C. Butler, M.D.

This protocol has been approved by the Host Defense Working Group

Signature of the Scientific Program Head T. C. Butler

Date June 21, 1984

7. ABSTRACT SUMMARY

Antigenic pattern of Vibrio cholerae belonging to both biotypes (classical and El Tor) and the serotypes (Ogawa and Inaba) will be analysed by crossed immunoelectrophoresis with rabbit antisera. Vibrio cholerae will be grown in different culture media and sonication will be used for preparation of antigens which will be further characterised. Pattern of specific antibodies in sera and intestinal washings of cholera convalescents will similarly be analysed using the antigenic preparations. Additionally, carbohydrate as well as protein antigens will be treated in mild alkali, acid and by acetylation to see if these modifications will not alter reactivity with antibodies present in the sera. Sera of healthy persons from

non-endemic areas will be used as controls. It is anticipated that comparison of antigenic spectrum obtained by means of the different sera will help identify the antigens which are presented to the host during the infectious process and as such should be considered as candidate for protective antigens incorporated into future vaccine.

8. REVIEWS

a. Research Involving Human Subjects _____

b. Research Review Committee _____

c. Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective:

The objective of this study is:

a) to analyse by technique of crossed immunoelectrophoresis which is known for its high resolution, specificity and sensitivity, the antigenic pattern of the vibrio component,

b) to compare the antigenic pattern of vibrio as reflected in the specific antibodies in cholera convalescents,

c) to determine the impact of the phenomenon of the phenotypic variations on presentation of defined antigenic components during the disease.

It is anticipated that information obtained from such study will help to determine antigenic components responsible for the acquired resistance and thus enable us to imitate natural process in respect of antibody production by incorporating the particular antigens into the vaccine.

2. Background:

Despite the immense efforts to immunize man against *Vibrio cholerae* infection in a safe and practicable way, no significant success has been reached. As stated by Rowley (1983), among several reasons adduced for this continuum of failures one may lay in liability of the protective antigens which are destroyed by the methods employed to prepare the vaccine used. This

problem appears to be even more complex in a view of the phenotypic variations in antigenic determinants which may occur in vivo and have not been mimiced in a process of vaccine preparation. Thus, we are not able to reach by vaccination the spectrum of specific antibodies which may reflect the level of immunity attained by natural V. cholerae infection. Obviously, a live oral cholera vaccine could mimic the natural process in stimulation of the immune response. However, no strain is available now which could be without limitations restricting its use as an living attenuated cholera vaccine. Though a significant progress was achieved in development of improved cholera vaccine (Holmgren et al. 1977), there has been a feeling that more research work is needed in this field. Oral vaccination, supported by the concept that mucosal immunity, both antibacterial and antitoxic nature, has been critically important in resistance to cholera has gained an attention (Svennerholm, Holmgren 1976). Subsequently, it has led to development of a combined oral vaccine consisting of B-subunit of the cholera toxin and killed whole cholera cells. While the B-subunit is relatively well-defined regarding the antigenicity, the whole cells represents a heterogeneous population of antigens. It is not known which of these play(s) a predominant role in the stimulation of immunity.

It has been known for sometime that substances presented on the surface of pathogen from the point of view of chemical composition and biological properties are not constant but are subject to phenotypic variations. Several examples may be cited. E. coli

isolated directly from urine of infected women possessed some membrane associated proteins different of those found after single colony isolation in vitro (Achtman, 1980). Recently Foo et al (1984) showed that porcine enterotoxigenic E. coli which in vitro reveal type 1 pili (F1) do not produce this type of pili in vivo in piglets. Kawaoka et al (1983) studied effect of the growth temperature on the properties of Yersinia enterocolitica lipopolysaccharide and found that LPS produced during growth in vivo resembles that produced during growth in vitro only at 25°C.

Such oriented studies only partly have been done with V. cholerae. Kabir (1980) showed that for instance the surface protein profile of V. cholerae depends on the medium of cultivation. When grown in the presence of peptone, a 15K protein band appeared; whereas, a 68K protein band appeared when Ogawa 395 was grown in the synthetic medium. Green (1982) working on growth pattern of V. cholerae in chemostat under carbon, nitrogen, magnesium and phosphate limitation showed that cholera vaccine of acceptable immunogenicity could not be produced. Important findings presented Attridge and Rowley (1983). They found a restricted distribution of non-LPS antigens among V. cholerae strains. Though the protective efficiency of antibodies against non-LPS components of the 569-B strain was much greater than that of antibodies to the lipopolysaccharide, authors doubt in protective value of the non-LPS components when applied orally due to a denaturation in the intestinal environment. These results, together with those published previously on effect of some environmental factors on

the antigenicity of gram-negative organisms (Formal et al 1956), Pirt et al 1961, Tempest and Ellwood 1969), fully supported the necessity to study V. cholerae antigenic determinants which stimulate production of specific antibodies under the environmental pressure of GI tract.

Studies in human volunteers have shown that specific immunity to cholera is indeed acquired following disease (Cash et al 1974), and it may last up to three years (Levine et al 1981). If this level of immunity could be achieved with a vaccine in the field, a significant progress could be made in cholera control. This, however, requires a good amount of knowledge about the antigenic determinants of V. cholerae, whether located in proteinaceous or carbohydrate components. Studies with animal models of cholera have brought some information. Watanabe et al (1968) using the active mouse-protection test showed that the protective antigen of an Ogawa strain was exclusively LPS in nature while that of an Inaba strain was a complex of LPS and heat-labile protein. Similar results were published by Eubanks et al (1977) who identified the protective component as a part of flagella. Also Neoh and Rowley (1972) showed that some protective antigens of protein nature may be involved in the expression of antibacterial immunity to the disease. However, it is necessary to mention that the detection and measurement of protective antibacterial and antitoxic antibodies depends on the qualitative as well as quantative serological tests. Several tests have been developed (Sack et al 1980, Horsfall and Rowley 1979). Recently Cryz et al

(1982) and Cooper et al (1983) developed for anti-V. cholerae antibodies ELISA originally described by Holmgren and Svennerholm (1973) for anti-LPS antibodies. However, none of these tests can qualitatively discriminate all specificities of antibacterial antibodies. Concerning anti-V. Cholerae antibodies in cholera patients, several studies were done on how infection is associated with a rise in titer of specific antibodies directed against cholera toxin (Pierce et al 1970), cell-wall lipopolysaccharide (Svennerholm et al 1976), outer membrane protein and cell-wall bound hemagglutinins (Foo et al 1981). Vibriocidal antibodies were estimated by Finkelstein (1962), Benenson et al (1968), Ahmed et al (1970) and vibriocidal reaction has been presently used for evaluation serological response to V. cholerae infection and vaccination (Clements et al 1982). However, the antigenic determinants in the preparation used in these reactions, except cholera toxin, were not precisely defined. This may be the reason for discrepancies described by Glass et al (1984) in vibriocidal and anti-LPS antibodies in population of endemic area of Bangladesh. While the vibriocidal antibodies were found to persist throughout life and could be associated with protection against colonization and disease, anti-LPS antibodies were of no additional or particular usefulness for protection. This is surprising since LPS is a prime antigen in vibriocidal reaction when studied in experimental conditions. The explanation of this findings may be:

a) other antigens than LPS take part in vibriocidal reaction; or

b) antigenic determinants of LPS involved in bactericidal reaction may be different of those present in LPS extracted by phenol-water procedure and used for anti-LPS estimation.

In fact, LPS of V. cholerae have revealed differences from those of the enterobacteriaceae: notably the absence of 3-deoxy-D-manno-octulosonic acid (d Ocl A) and the presence of fructose and unusual aminosugar (Broady et al 1981). Side chains which may carry main antigenic determinants were shown to consist of a regular α (1 - 2) linked chain of D-4-amino-4, 6-dideoxy-D-manose in V. cholerae Inaba serotype. O-side chains of Ogawa serotype have 4-amino-4-deoxy-L-arabinose as an additional component (Redmond 1978, 1979). In these structural components, the amino functions are acylated with 3-hydroxypropionyl groups. Though it is known that the somatic antigens do distinguish V. cholerae Ogawa, Inaba and Hikojima through AB, AC and ABC specificities (Greenough 1979), an exact location of these determinants in the above-mentioned structures is not known. Interestingly, the serotype shifts between Ogawa and Inaba occur in nature and in vitro (Barua 1974) but to what extent are related to alternations in outer membrane structural components it is not known. From previous studies, it has been known that the antigenic activity is determined by the monosaccharide, the type of linkage and the presence of substituents. If any of these determinants is removed or added, the antigenic pattern is changed as it was shown in the case of S. typhimurium LPS (Ciznar, Shands 1970). If conditions

for removing or alteration of the determinants can occur in vivo during the process of adhesion and subsequent colonization of the host intestinal surface by V. cholerae, then the result would appear in the spectrum of specific antibodies. Such antibodies should be, in a case of repeated infection, very effective in preventing the association of bacteria with intestinal mucosa and subsequent colonization due to their specific interaction with modified bacterial cell surface. If this mechanism would operate in vivo, it seems obvious that antibodies formed after administration of vaccine in which no such conditions were respected during preparation would only partly be active in preventing the association and colonization, though effective enough in serological tests with corresponding antigens.

3. Rationale:

Vaccination against cholera remains, in many developing countries, only approach to prevent cases and ameliorate severity of the disease. This is due to the fact that establishment of proper sanitation and supply of safe water and food cannot be presently achieved because of high cost and socio-economical and cultural status. However, the development of an effective immunizing agent against cholera has still been facing the main problem: which of the cell structures or products carry the protective antigens, and antibodies against which of these structures serve as the mediators of resistance against cholera? Identification of these structures appears to be a prerequisite in construction of an effective cholera vaccine through the gene-hybridization

technology or synthesis of relevant antigens or classical preparation of the vaccine. The proposed study employing sensitive and specific crossed immunoelectrophoresis intends to characterise such antigens which may be presented only in vivo under the environmental pressure of the intestine, and therefore, should be reflected in the spectrum of specific antibodies. In order to detect them, only empirical approach, comprising various cultivation procedures and exogenous modifications of the cell structures are likely to be helpful in this task.

B. SPECIFIC AIMS

1. To identify antigens of V. cholerae by the pattern revealed in crossed immunoelectrophoresis with rabbit antiserum and to find a medium in which maximal number of antigenic determinants are produced.

2. To study the phenomenon of phenotypic variations in antigenic determinants using various parameters, such as, the effect of media, growth kinetics and exogenous factors as mild alkali and acid-hydrolysis and acetylation and relate it to the pattern revealed by crossed immunoelectrophoresis.

3. To analyse pattern of antibodies in sera and intestinal washings of cholera patients by means of antigenic preparations already tested with rabbit antisera.

4. To identify components responsible for stimulation of antibody production in cholera patients.

C. EXPERIMENTAL

Bacterial strains

Vibrio cholerae of both biotypes and serotypes will be included in the study. Laboratory stock strains will be used in the first part of the study with rabbit antisera. For analysis of human sera and intestinal washings homologous strains will be used.

Media

Strains will be cultivated in 3% solution of peptone water enriched by yeast extract (0.5%), pH 7.4. Semisynthetic medium will be used according to the procedure described by Finkelstein et al (1966). Synthetic medium will consist of the same ingredients as semisynthetic one, except Casamino acids. Moreover, aminoacids glutamin, serin, aspartic acid and arginin will be added in concentration 0.25%.

Immunogen preparation

For the immunization of rabbits cells grown in different media will be sonicated at 20 K Hz for 10 minutes. Colloid concentration corresponding to 10^8-10^9 cells will be used for immunization of rabbits.

Crossed immunoelectrophoresis: A modification with or without intermediate gel will be carried out as described by Kroll (1973). Briefly, barbitat-Hcl buffer (ionic strength 0.02, pH, 8.6) containing 1% (vol/vol) Triton x-100 will be used and incorporated into 1% agarose gel. The first dimensional separation will be carried out at 10 V/Cm on a plate of 5 x 5 cm and bromphenol

blue-labeled human albumin will be used as a marker. Second dimension electrophoresis will be carried out at 3 V/Cm for approximately 18 hours. The gel will contain 18 μl of antiserum per cm^2. Antigens in concentration 8-18 $\mu l/cm^2$ will be incorporated into intermediate gel for crossed-line immunoelectrophoresis. After electrophoresis, the gel will be washed, pressed, dried, stained and destained by the usual procedure.

The following procedures will be undertaken to identify the nature of antigens and corresponding patterns in immunoelectrophoresis.

Soluble components of the sonicated preparation will be separated by gel chromatography using Sephadex and Sepharose gels and by FPLC system. The homogenety of individual fractions will be determined by PAGE electrophoresis and tested in crossed immunoelectrophoresis. Extract of sonicated cell suspension will be tested for presence of thermolabile antigens by heating to $100^{o}C$ for 20 minutes.

Proteinaceous antigens will be separated from the sonicated extract by TCA precipitation. The content of proteins and carbohydrates in the samples will be determined by standard procedures of Lowry (1951) and Morris (1948).

Proteins of outer membranes will be isolated by the method of Schnaitman (1971) and of Kelley and Parker (1981).

LPS will be isolated by the procedure of Westphal and Jann (1965).

Flagella preparation: Crude flagella will be prepared by the method described by Resnich et al (1980). Contamination of flagella preparation by LPS will be estimated by a Limulus lysate assay.

Modifications of antigens determinants will be carried out for various length of time under mild acidic conditions (20 m Macetate buffer, pH 4.4) and mild alkali conditions (0.2 - 0.1 M Na OH) as well. Acetilation will be done by means of acetic anhydrid in the presence of $NaHCO_3$.

Animal sera: Rabbits will be immunized with immunogen dispersed in Freund incomplete adjuvant. Intraperitoneal injections of immunogen corresponding to 10^8, 5.10^8, 10^9, 5.10^9, 10^{10} cells will be applied in weekly intervals. During the immunization schedule, a blood sample will be taken after 3 and/or 4 dose and the response will be tested by a bacterial agglutination. Sera with titer $\geq$1/5,120 will be further used.

Human sera. Twelve male patients 18-40 years old, presenting to ICDDR,B treatment centre, with history of accute watery diarrhoea (duration less than 12 hours) and bacteriologically confirmed cholera (moderate or severely dehydrated) will be included into the study. No prior antibiotic treatment is allowed, except when indicated by the clinical condition of the patients. Patients will be rehydrated with I.V. fluids (acetate) and maintained hydration with I.V. Intake and output will be measured every 8 hours and body weight taken daily. Cultures will be made directly from stool on the 1, 3,

5 and 8 day from admission. Informed written consent will be obtained. On the 10 day of hospitalization, a sample of blood will be taken from each patient. The serum in volume of 7 ml obtained from the samples will be aliquoted and frozen at -40°C. Concentration of serum sample on one plate will be 20 $\mu l/cm^2$. Using plates 5x5 cm this corresponds to 0.5 ml per one analysis. Totally, 12 analysis will require 6 ml of sera. One ml of sera will be kept for repeated analysis.

The lavage specimen will be collected from the cholera patients by the procedure which has been used in ICDDR,B since 1979. The volume 1000 ml of watery stool will be heated at 56°C for 15 minutes to inactivate proteolytic enzymes then sterile-filtered and concentrated on ultramembrane to 20 ml. The concentrated material will be used in crossed immunoelectrophoresis analysis.

D. SIGNIFICANCE

The proposed protocol after successful completion will bring information about the number and nature of V. cholerae antigens which might be involved in stimulation of antibacterial immunity during the natural V. cholerae infection. Particularly important appear to be the information:

a) How many phenotypic variants of V. cholerae Ogawa and Inaba serotypes may be presented in environment in vitro.

b) How many antigens are presented by V. cholerae and reflected in serum and intestinal antibodies during the infection leading to an acute watery diarrhoea.

c) Whether any modification known to alter the position of immunodeterminant groups on carbohydrate moiety of LPS has been reflected in the specific antibodies.

Information which may arise out of these experiments, together with the fact that specific immunity to cholera is indeed acquired following disease, could suggest which antigenic component of vibrio are responsible for the acquire resistance and, therefore, to be incorporated into the immunizing agent. Moreover, the particular antigens could be used in future seropidemiological studies oriented to determination of antibodies as indicator of infection and resistance.

The field trial with whole-cell/B-subunit cholera vaccine, which will be conducted at ICDDR,B, will offer an opportunity to compare the spectrum of V. cholerae antibodies against the antigens detected by above mentioned procedure in vaccinated persons and convalescents and relate them to the level of immunity afforded by vaccination and natural infection.

E. FACILITIES REQUIRED

Laboratory space - Bench space for working of PI and Co-PI will be needed. Laboratory No. 106 on the first floor will be adapted for the experimental work. Equipments for analysis and separation of antigens will be located in this laboratory. For separation purposes, FPLC system of Pharmacia Fine Chemicals, Sweden, will be used.

F. COLLABORATIVE ARRANGEMENT

This protocol was discussed in details with Professor Dr. D. Rowley. His research team will provide material and technical help, and comparative analysis will be done using data from crossline immunoelectrophoresis performed at ICDDR,B and from Western blot technique performed at Department of Microbiology and Immunology, University of Adelaide. An arrangement has been made with Dr. G. Bartkova, Research Institute of Preventive Medicine, Bratislava, Czechoslovakia, who will supply us with sera of healthy blood donors and will also analyse immunochemically the phenotypic variants.

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	Time Effort	Project Requirement Taka	Project Requirement Dollar
Dr. I. Ciznar	Investigator	40%	-	30,000
Dr. A. Khanam	Co-investigator	10%	3,500	-
Dr. A. Ahmed	Co-investigator	5%	7,650	-
Dr. D. Sack	Co-investigator	5%	-	3,500
Senior Research Officer	To be named	50%	36,000	-
Laboratory Technician	To be named	50%	14,000	-
Assistant Animal Caretaker	To be named	5%	1,200	-
			62,350	33,500

2. SUPPLIES AND MATERIALS

	Unit Cost	Amount	Project Requirement Taka	Project Requirement Dollar
- Plastic, glassware (plates, dialysis bags, tubes, flasks, pipets)				3,500
- Reagents, chemicals, drugs media				3,000
- New Zealand adult Albino rabbits	140	35	4,900	
			4,900	6,500

3. EQUIPMENT

Separation unit of Pharmacia Fine Chemicals FPLC				10,000

		Project Requirement	
		Taka	Dollar
4.	HOSPITALIZATION COSTS		
	150 patient days	22,500	
5.	OUTPATIENT CARE		
	Nil		
6.	ICDDR,B TRANSPORT		
	Nil		
7.	TRAVEL AND TRANSPORTATION OF PERSONS		
	Local : Nil		
	International: Round-trip travel Dhaka-Adelaide-Dhaka		2,500
8.	TRANSPORTATION OF THINGS		250
9.	RENT, COMMUNICATIONS AND UTILITIES		
	Postage		100
10.	PRINTING AND REPRODUCTION	10,000	
11.	OTHER CONTRACTUAL SERVICES		
	Patient fees	2,000	
12.	CONSTRUCTION		
	Nil		

B. BUDGET SUMMARY

		Project Requirement	
		Taka	Dollar
1.	PERSONNEL SERVICES	62,350	33,500
2.	SUPPLIES AND MATERIALS	4,900	6,500
3.	EQUIPMENT	-	10,000
4.	PATIENT HOSPITALIZATION	22,500	-
5.	OUTPATIENT CARE	-	-
6.	ICDDR,B TRANSPORT	-	-
7.	TRAVEL AND TRANSPORTATION	-	2,500
8.	TRANSPORTATION OF THINGS	-	250
9.	RENT, COMMUNICATIONS AND UTILITIES	-	100
10.	PRINTING AND REPRODUCTION	10,000	-
11.	CONTRACTUAL SERVICES	2,000	-
12.	CONSTRUCTION	-	-
		100,750	52,850
		US$ 4,030	

TOTAL ...	US$56,880
SALARIES ...	US$35,994
OPERATIONAL COST ...	US$20,886
CONVERSION RATE : US$ 1 = Taka 25	
INCREMENTAL COST : 10% of US$ 56,880	US$ 5,688
GRAND TOTAL	US$62,568

ABSTRACT SUMMARY

The purpose of the proposed study will be detection and discrimination of Vibrio cholerae antigens which are responsible for stimulation of antibody production during the infectious process and which might be different of those presented in vitro. Such phenotypic variants can be detected by means of specific antibodies present in sera or intestinal content of cholera convalescents. Crossed immunoelectrophoresis with or without intermediate gel will be used for the analysis. Modifications of carbohydrate determinants will be carried out in order to simulate in vivo conditions for presentation of antigens. Such antigens may be potential candidates for vaccine stimulating immunity equivalent to that one attained by natural V. cholerae infection.

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

2. The risks from this study are minimal and consist of the discomfort during taking blood sample.

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.

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 (enclosed).

6. N.A.

7. The individual will gain no personal medical benefit other than treatment for cholera which he would receive regardless of the study. Society will benefit if this study can give more information on protective antigens of V. cholerae and thus improve efficiency of the vaccination.

8. Blood and intestinal washings will be collected.

CONSENT FORM

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 occurs when a person develops cholera. We hope that the information we gain will be helpful in developing more effective cholera vaccine. 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.

2. On the last day of hospitalization, we will take a sample of blood in volume 15 ml. Also we will have you do the intestinal lavage procedure. 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.

3. None of the tests are harmful to your health. Drawing blood and drinking a large volume of salty water are somewhat uncomfortable; they do not have any serious side-effects.

4. Your medical records will be kept confidential.

5. You do not have to participate in the study. Your decision concerning the study will not affect 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

সম্মতি পত্র

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ কলেরা এবং অন্যান্য ডায়েরিয়া রোগের বিরুদ্ধে প্রতিরোধ ক্ষমতা গড়ার গবেষণা চালাচ্ছে। আমরা কলেরা রোগে আক্রান্ত রোগীর রোগ প্রতিরোধ ক্ষমতা নির্ণয়ের গবেষণায় আপনাকে অংশগ্রহণের অনুরোধ জানাচ্ছি। আমরা আশা করি এই পরীক্ষার ফলাফল অধিকতর কার্যকরী কলেরা ভ্যাকসিন তৈরীর কাজে বিশেষ সহায়ক হবে। আপনি এই পরীক্ষায় অংশগ্রহণে রাজী থাকলে নিম্নের পরীক্ষাগুলো করা হবে :

১। আপনাকে হাসপাতালে ১০ দিন থাকতে হবে।

২। চিকিৎসার শেষ দিনে আপনার কাছ থেকে ১০ মিলিলিটার রক্ত নেওয়া হবে। আমরা আপনার অন্ত্ররস পরীক্ষা করতে চাই। এজন্যে আপনাকে প্রচুর লবনাক্ত তরল পদার্থ (প্রায় পাঁচ সের পর্যন্ত) খেতে হবে। এর ফলে আপনার সাময়িক ডায়েরিয়া দেখা দেবে। এই লবনাক্ত তরল পদার্থ খাওয়া শেষ হবার সাথে সাথেই আপনার ডায়েরিয়াও কমে যাবে। এই প্রক্রিয়ায় আপনার মনে হবে আপনার পেট ভরা, আপনার ওজন ১ থেকে তিন সের বেড়ে যেতে পারে, কিন্তু আপনার কোন পেট ব্যাথা বা সাংঘাতিক পার্শ্বপ্রতিক্রিয়া দেখা দেবে না।

৩। কোন পরীক্ষাই আপনার স্বাস্থ্যের জন্য ক্ষতিকর নয়। রক্ত নেবার সময় বা প্রচুর লবনাক্ত তরলপদার্থ খাবার সময় একটু অসুবিধা হলেও তাদের কোন খারাপ পার্শ্বপ্রতিক্রিয়া নেই।

৪। আপনার চিকিৎসা সংক্রান্ত নথিপত্র গোপন রাখা হবে।

৫। পরীক্ষায় অংশগ্রহণ করা বা না করার সাথে এই হাসপাতালে আপনার চিকিৎসা নির্ভরশীল নয়। যদি আপনি এই গবেষণায় অংশগ্রহণ করেন তবুও আপনি যে কোন সময় চলে যেতে পারবেন, এতে করে আপনার চিকিৎসার কোন ক্ষতি হবে না। এই গবেষণার ব্যাপারে আপনার যে কোন কিছু জানার থাকলে আমরা আপনাকে জানাব।

আপনি এই গবেষণায় অংশগ্রহণ করতে রাজী থাকলে আপনার সহি বা টিপসহি এখানে দিন।

স্বাক্ষর/টিপ সহি

গবেষক

তারিখ----------