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CE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

ncipal Investigator DR. S. A. SARKER Trainee Investigator (if any) _____

lication No. 93-020 Supporting Agency (if Non-ICDDR,B) _____

le of Study Development of Non-Invasive Tests to Assess Gastric Acid Output in Children.

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

cle 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:
___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
___ Informed consent form for subjects
✓ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
✓ Questionnaire or interview schedule *

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

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

S A Sarker
Principal Investigator

Trainee

ENTERED 2 1 JUN 1993

TITLE	:	DEVELOPMENT OF NON-INVASIVE TESTS TO ASSESS GASTRIC ACID OUTPUT IN CHILDREN
PRINCIPAL INVESTIGATOR	:	DR. S. A. SARKER
CO-PRINCIPAL INVESTIGATOR	:	DR. D. MAHALANABIS
CO-INVESTIGATORS	:	DR. P. K. BARDHAN LAB. RESEARCH OFFICER (To be named)
CONSULTANTS	:	DR. K. S. RABBANI DR. ADNAN KIBOR DEPARTMENT OF PHYSICS, UNIVERSITY OF DHAKA PROF. R. B. SACK, ICDDR,B.
COLLABORATING INVESTIGATOR:		PROF. KLAUS GYR UNIVERSITY OF BASEL, SWITZERLAND
STARTING DATE	:	AS SOON AS POSSIBLE
STUDY DURATION	:	1 YEAR
TOTAL DIRECT COST	:	US $ 25,950.00

te:_____

Signature
Associate Director
Clinical Sciences Division

1. Main Objective

(1) To validate two non-invasive tests (i) Applied potential Tomography (APT) and (ii) Titratable urinary acid output, to assess gastric acid secretion against the "gold standard" pentagastrin test.

2. Abstract Summary

Although gastric acid is considered to be an important non-specific defence against infectious diarrhoea, field studies of the role of gastric acid in preventing enteric infections has been hampered by the lack of a suitable non-invasive test. Since low gastric acid is an important risk factor in infectious diarrhoea and since diarrhoea is one of the major causes of mortality and morbidity in infants and young children, it would be important to discriminate between children at high and low risk of developing infectious diarrhoea. We propose to validate two non-invasive tests to assess gastric acid output against the "gold standard" pentagastrin test.

The study is designed firstly to measure tomographic images of the resistivity of gastric contents using electrodes placed around the upper abdomen and secondly, to quantify changes in urinary acid secretion in basal and stimulated conditions and relate them to changes in gastric acid secretion in those conditions. The study will be performed at ICDDR,B's Clinical Study Ward and will be conducted initially on 10 healthy adult volunteers. Gastric resistivity changes and urinary titratable acid excretion during basal and stimulated conditions will be compared with corresponding acid outputs of the pentagastrin test. If the results are found to be encouraging in adults, the study will be extended to seventy children of 6 months to 3 years of age convalescing from acute diarrhoea.

If the two non-invasive methods are found to correlate with the standard pentagastrin test, they may be useful for clinical evaluation and epidemiological screening for low gastric acid secretory state, and may indicate those who are at a risk for developing infectious diarrhoea.

3. Introduction:

That gastric acidity plays a key role as a barrier against infectious diarrhoea has been recognised since 1932 (Hurst 1993). The very endemicity of infectious diarrhoea in the third world countries (Cook 1985) may be related to a higher prevalence of low gastric acid secretion. In fact, low gastric acid secretion has been found to be linked with an increased susceptibility of *Cholera* and *Escherichia coli* diarrhoea in adults (Cash 1978, Gianella et al 1973, Sack et al 1972, Nalin et al 1978, Gilman et al 1981, Van Loon et al, 1991) in those countries.

Information on gastric acid secretory states in children is scanty. A decreased gastric acid secretion and its association with intragastric bacterial colonization has been reported in Bangladeshi children with

protein-energy-malnutrition (Gilman et al. 1988). Since infectious diarrhoea is one of the leading causes of childhood mortality and morbidity, and since malnutrition and associated infectious diseases are common childhood affliction in many parts of the world, it would be important to know if decreased gastric acid secretion in children increases the risk of infectious diarrhoea. Unfortunately, studies to see the role of gastric acid in infectious diarrhoea particularly in children has been limited by the lack of suitable non-invasive test. The standard acid secretion test requires nasogastric intubation for gastric aspiration and administration of a stimulant which may be unpleasant to the patients and sometime hazardous. Several tube-less tests have been evaluated to assess gastric acid secretion in the past. Most of the tests are qualitative and indirectly determine gastric acid secretion, e.g. by means of resin dyes (Christiansen, 1966), radiotelemetry capsules (Andres et al, 1970), radioisotopes (Taylor et al, 1979) and serum group I pepsionogen (Samloff et al, 1975). But the tests were not found simple, suitable and accurate for assessment of gastric acid secretion, and they involve the use of radioactive tracers in some of the tests (^{99}Tc). Oral Magnesium Breath Hydrogen Test (OMBHT) (Sack et al 1985, Stephensen et al 1987, Sarker, 1991)also estimate gastric acid output by measuring breath hydrogen excess after ingestion of magnesium and stimulant of gastric acid. But the test was found to underestimate acid output in comparison to standard test for gastric analysis in some of the study subjects. More recently, titrable urinary acid excretion as an index of gastric acid output has been proposed as a non-invasive alternative to conventional gastric secretion studies (Johnson 1990). Impedence imaging with Applied Potential Tomography (APT) is another tube-less approach which has also emerged as a simple, non-invasive method for assessment of gastric acid output in healthy volunteers and patients with peptic ulcer diseases (Mangnall et al, 1987; Baxter et al, 1988). The aim of this study is to validate these two non-invasive tests (Titratable Urinary Acid excretion and Applied Potential Tomography) against the standard pentagastrin test in children convalescing from diarrhoea.

4. Specific Aims:

i) To validate acid secretion as estimated by Applied Potential Tomography (APT) and titratable urinary acid excretion against the Pentagastrin test (PGT).

ii) To determine the gastric acid secretory states in children convalescening from acute diarrhoea.

5. BACKGROUND

The following points are considered as justification of such a study:-

* Epidemiology of hypochlorhydria
* Infective gastroenteritis, persistent diarrhoea and their relationship with reduced gastric acidity.

* The gastric acid barrier in malnutrition
* The importance of hypochlorhydria in the Third World Countries

5.1. Epidemiology of hypochlorhydria

The prevalence of hypochlorhydria varies widely, but is high in the third world countries(Cook et al 1985). Severe malnutrition in infancy can certainly predispose to hypochlorhydria (Gracy et al 1977, Thomason et al 1980). A reduced acid output as measured by the augmented histamine test has been demonstrated in most malnourished children in Indonesia (Gracy et al 1977), Nigeria (Adesola et al 1968), South Africa (Witman et al 1968) and Bangladesh (Gilman et al 1988). Relative hypochlorhydria has been demonstrated to occur in the first week of life and in elderly people over the age of sixty (Wadell et al 1956) and thus might explain the increased incidence of small bowel colonization as observed in infants (Agunod et al 1969) and the increased risk for Salmonella infection in the elderly people (Wadell et al 1956, Gorbach et al 1983, Holt et al 1989). A high intragastric pH has also been observed in people of countries in Africa, South America and Central America and possibly southern and eastern Europe (Hill et al 1985). On the contrary, an acidic and sterile stomach is normal in the United States and north western Europe. These observations suggest environmental contamination and a possible association with infectious agents as being the cause of hypochlorhydria in the third world countries. Till now, information on the gastric secretory state in infectious diarrhoea in children is lacking. Hypochlorhydria has been demonstrated in adult patients convalescing from cholera in Bangladesh. (Nalin et al. 1978, Vanloon et al. 1991) and India (Sack et al 1972). The information on acid secretary states in children may be particularly important in designing appropriate diarrhoeal intervention programmes in this population.

5.2. Diarrhoea and gastric acidity

5.2.1. Infective gastroenteritis and its relationship to gastric acidity

Hypochlorhydria, by compromising the defense mechanisms of the upper gastrointestinal tract, predisposes to intestinal bacterial and parasitic infections. One possible serious consequence of achlorhydria or hypochlorhydria was suggested by the eminent British gastroenterologist Sir Arthur Hurst in 1934, when he mentioned the possibility that "bacillary and amoebic dysentery occur much more commonly in people with achlorhydria and hypochlorhydria". He further speculated that the same might be true for typhoid fever and cholera (Hurst 1934). The earlier observation made by Sir Arthur Hurst has been confirmed by a series of studies demonstrating hypochlorhydria as a predisposing factor to the growth of organisms responsible for typhoid and non-typhoid salmonellosis (Giannella et al 1971), bacillary dysentery (DuPont et al 1972), giardiasis (Anonymus 1982), cholera (Nalin et al 1978, Sack et al 1972) and other infectious agents responsible for gastroenteritis (Cook 1985).

Another report suggests that Clostridium difficile infection (pseudomembranous colitis) may also be associated with hypochlorhydria (Gurian et al 1982). Whether, hypochlorhydria as observed resulted from the effect of bacterial toxins or preceded the infection is not yet established. However Nalin et al (1978) demonstrated that cholera did not alter gastric acid secretion in a group of American volunteers. On the contrary hypochlorhydria, as observed in convalescing cholera patients in Bangladesh by the same author, indicates that hypochlorhydria might have preceded the infection (Nalin et al 1978).

Among parasitic infections, the most impressive evidence relates to giardiaisis (Hass & Becken 1967) and strongyloidosis (Jones 1950). In some instances, the infections themselves may also cause hypochlorhydria; longitudinal studies are required to answer this question clearly. The importance of hypochlorhydria in 'third world' populations in whom gastrointestinal infections are extremely common, particularly in infants, needs to be evaluated.

5.2.2 Persistent diarrhoea and hypochlorhydria

Persistent diarrhoea, at present is an emerging problem in third world populations. It has been reported that as many as 15% of all acute diarrhoeal episodes that begin acutely last beyond 2 weeks (Glimpse, ICDDR,B 1992). Studies from Bangladesh and India have shown that 30-52% of diarrhoeal deaths are associated with persistent diarrhoea (Gorbach et al 1986, Bhan et al 1986, Fauvaeu et al 1991). Information on risk factors for persistent diarrhoea is scanty. It is probable that acute gastroenteritis in some way alters host factors which increase the risk of persistence of subsequent diarrhoeal episodes. An association between recent diarrhoeal illness and an increased risk of persistent diarrhoea has been observed in India (Sazawal et al, 1992) and in Bangladesh (Baqui et al, 1992).

A study at ICDDR,B provided evidence that pathogens isolated during the acute phase of diarrhoeal illness in children are as a rule different from those isolated from the same children when diarrhoea lasted beyond 14 days (PhD thesis, John Hopkins University, Baqui et al 1991). The very different organisms involved in persistent diarrhoea could be related to qualitative and quantitative alterations of gut flora as occurs following Enterotoxigenic E. coli infection (Eastwood 1977). It is more likely that alteration of the gastric acid barrier that may have preceeded or may be associated with acute gastroenteritis, might have encouraged colonization of the abnormal bacterial flora in the mucosa of the intestine and thus have led lead to persistent diarrhoea. Defective gastric acid secretion was considered to be a predisposing factor to abnormal bacterial overgrowth and recurrent enteric infections (Howden 1987) both conditions that may lead to the development of persistent diarrhoea and malabsorption(Mata et al 1972, Cook et al 1986, Omoike et al 1989,)

resulting in malnutrition and growth failure. Hypochlorhydria and achlorhydria have been observed in 55% of patients with tropical sprue (Vais et al 1965).

Higher risks of prolonged diarrhoeal illness in infancy are observed in India (Bhan K 1989, Sazawal 1992) and Brazil (MCAuliffe 1986). The risk of prolonged diarrhoeal illness in this age could be related to physiological immutarity in the gut immune system. However, the role of the gastric acid barrier in modifying the course of diarrhoea cannot be ruled out in this particular age group. Development of a suitable non-invasive technique to assess gastric acid output would thus be important in designing studies to find out the role of gastric acid in acute and persistent diarrhoea in children.

5.3. Gastric acid barrier in malnutrition

The gastric acid barrier was found to be impaired in malnourished children and protein-depleted experimental animals (Thomason et al 1980, Gracy et al 1977, Wittman et al 1968). The effect of malnutrition on gastric acidity and gastric bacterial colonization was studied in 35 severely malnourished Bangladeshi children (Gilman et al 1988). Gastric acid outputs, both basal and after betazole stimulation, was found to be significantly lower in malnourished children compared to that in better nourished children. Hypochlorhydria was accompanied with gram negative bacterial colonization of gastric juice in 81% of the malnourished children, whereas none of the better nourished children had gram negative colonization. After 3 weeks of nutritional rehabilitation, although gastric acid secretion was found to have increased, the gastric acid concentration still remained depressed in malnourished children. Hypochlorhydria was again found to be accompanied with gastric colonization. The factors associated with decreased acid output in those children remain unclear. Although anaemia was postulated to be associated with reduced gastric acid output (Coowan et al 1966), control studies have not been performed. However the low gastric acid output may be related to chronic gastritis, found in the biopsy specimens from the gastric fundus of malnourished children (Gracy et al 1977, Wittman et al 1968). No data are available on parietal cell numbers and their changes upon rehabilitation in malnourished children. The prolonged depression in acid concentration as observed in the study with Bangladeshi children suggests that nutritional support for a period longer than three weeks is required to effect full reconstitution of acid secretion.

5.4. Importance of hypochlorhydria in the third world population

Both reduced acid secretion and infectious diarrhoea occur with high frequency in third world countries. Malnutrition predisposes to chronic gastritis and reduced acid secretion in those populations. At present, the importance of hypochlorhydria in those countries is impossible to assess because of lack of suitable non-invasive test to assess acid secretion.

Malnutrition in infancy can certainly predisposes to hypochlorhydria as reported in Indonesia (Gracy et al 1977) and in Bangladesh (Gilman et al. 1988). It also seems clear that hypochlorhydria predispose to infections with *V. cholerae, Shigellae*, nontyphoid *Salmonellae* and Giardia in these populations. Because of the poor environmental conditions in developing countries, people are very often exposed to these enteric pathogens. Low gastric acid production has been found to be associated with high risk of Cholera and E. coli diarrhoea in those communities (Scrimshaw et al 1968, Sack et al 1972). Recently H. pylori infection has been found to be associated with hypochlorhydria (Morris et al. 1987, Graham et al. 1988). It is possible that H. pylori associated hypochlorhydria leads to increased susceptibility to enteric pathogens in those population.

6.1 Subjects

We will conduct the study initially on 10 adults healthy volunteers of either sex 18 to 30 years of age. If the results are found to be encouraging the study later on will be conducted on 70 convalescing children of 6 month to 3 years attending the outpatient department of ICDDR,B with a history of acute watery diarrhoea of less than 5 days duration. A random sample of those who stayed in the observation ward for more than 48 hours during which cessation of diarrhoea has been confirmed by passage of soft stool, will be included in this study. Informed consent of the childrens' parents will be obtained after the study procedures are explained in detail to them.

6.2 Methods and Procedures

All the subjects will be admitted to the study ward of the Clinical Research Centre of ICDDR,B,. A clinical examination will be performed. The usual hospital diet will be provided. Breast-fed child will be allowed to have their mother's milk. All of them will be kept in hospital for 2 days. In children, daily stool volume, consistency and intake-output records will be recorded under close supervision to ensure lack of diarrhoea.

The following tests will be performed during the 2 days of hospitalisation.

1. Pentagastrin test
2. Applied Potential Tomography (APT)
3. Measurement of Titratable urinary acid excretion.

APT and measurement of titratable urinary acid excretion will be done simultaneously in one day. The pentagastrin Test will be done on a separate occassion. However, the sequence of the tests (pentagastrin test or APT and titratable urinary acid excretion) will be chosen at random.

6.3 Exclusion Criteria:-

* Children with severe protein energy malnutrition <60 weight for age of the median of National Center for Health Statistics (NCHS).
* Presence of systemic illness e.g. pneumonia, septicaemia, urinary tract infection and otitis media.
* Children who had recent virus infection, e.g. measles as evident by typical history and rash.

6.4 Tests of Gastric acid determination

6.4.1. Pentagastrin Test (Baron et al 1978)

After a period of fasting (overnight for adults and 4 hours for children) a flexible gastric tube (Levin tube 21-23 F in children, 14-16 F in adults) lubricated with water soluble vegetable gel will be swallowed by the study subjects in the following morning after admission in the study ward. The tip of the tube will be placed in the most dependent part of the stomach. The position of the tube will be confirmed by auscultation, aspiration of gastric juice and nearly complete recovery of water injected through the tube. Samples of gastric juice collected during the first 10 minutes will be discarded. After aspirating all residual gastric juice, basal gastric juice will be collected continuously for half an hour. The secretions will be separated into 15 minute collections.

Pentagastrin (Peptavalon, ICI) will then be administered subcutaneously at a dose of 6 µg/kg body weight. After a period of 10 minutes following injection, aspiration of gastric juice will then be resumed for another 1.5 hour, again separated into 15 minute period.

In children, to avoid the possibility of hypoglycaemia, fluid containing 10% dextrose will be infused intravenously during the period of fasting and the tests.

Gastric juice samples will be collected from children and adults lying in the left recumbent position using manual suction from the tube. The volume of each 15 minute sample collected will be measured to the nearest 0.5 ml. The pH will be measured with a glass electrode adjusted for ambient temperature. The concentration of acid will be determined by titration with 0.01 N NaoH to pH 7.0 with an automatic titration unit (Metrohm AG, Switzerland). The acid output will be expressed as mmol/hour.

Calculation : Total acid output (mMol HCl/Hour) is the product of the concentration of acid in the gastric juice (mMol HCl/l) and the total volume of the gastric secretion (L) following pentagastrin administration. Similarly Basal Acid Output (BAO) is the product of acid concentration and the total volume of the basal secretion.

6.4.2 **Applied Potential Tomography** (Mangnall et al 1987, Baxter et al 1988). Applied Potential Tomograpy (APT) which has also been termed conductivity imagining and electrical impedence imagining, is a new, non-invasive technique that generates tomographic images of tissue resistivity from measurements made from multiple electrodes placed around the upper abdomen. The technique allows multiple studies to be carried out on the same patient and is sufficiently postuleted to be used in a hospital ward. The system consists of a data collection unit used to measure and process the signals from electrodes placed around the abdomen and a microcomputer which reconstructs the APT images. The images are displayed on a screen and data may be copied to a printer for patients records.

6.4.2.1 Principle of the test:

Secretion of acid into the stomach alters ionic conductivity (resistivity) which can generate tomographic images which can be measured by APT using electrodes placed around the abdomen.

Method

To record tomographic images of tissue resistivity by APT, sixteen electrodes will be evenly placed in a circle around the upper abdomen at the level of the 8th costal cartilage. An alternating current of 1 mA at 50 KHz will be passed between two adjacent electrodes and the potential gradients at the remaining 13 pairs will be measured. For example, when current is passed between electrode 1 and 2, then the potential difference between 3 and 4, 4 and 5 and so on upto 15 and 16 will be recorded. Each pair of electrodes in turn, will act as the drive electrodes. For a 16-electrode assay, 208 measurements will be made during each cycle lasting 100 ms. A number of cycles will be averaged to form one data set. Electrode contact will be monitored before and after completion of data collection of the study.

After the data is processed and displayed as an image a fixed line Region of Interest (ROI) will be outlined corresponding to the stomach. Any subsequent data set will be then back projected against the initial set to produce a cross sectional image of the changes in the distribution of resistivity in the area of the electrodes. Results will be expressed as percentages of the maximum change obtained. Following an initial reference image, one-minute frames will
be collected for 30 minutes, to estimate basal line resistivity (R-Basal) of the stomach.

After 30 minutes of recording of baseline resistivity (R-Basal), Pentagastrin will be administered 6 µg/kg body weight. After 10 minutes of pentagastrin one minute frames will be collected for another 60 minutes after pentagastrin administration (R-Maximum). The maximum change in resistivity in the region of interest (ROI) before (R-Basal) and

after pentagastrin injection (R-Maximum) will be obtained. The basal acid output (BAO) as assessed by PGT will be compared with R-Basal and the total acid output will be compared with R-Maximum. Figure of portable unit (enclosed at the end of the protocol).

6.4.3. Measurment of Titratable Acid Output in Urine

6.4.3.1 Principle of the test: During secretion of acid by the gastric mucosa bicarbonate diffuses from parietal cells into the blood (Ganong 1986). Excess bicarbonate is excreted by the kidney, which means that a transient rise in urine bicarbonate excretion (the urine "alkaline tide") immediatedly follows maximal gastric acid secretion. This acid change (alkalinisation) in urine reflects changes in gastric acid secretion.

6.4.3.2 Collection of urine samples and chemical determination.

In children, urine samples will be collected by application of pediatric urine collection (PUC) bag. The adult volunteers will be asked to collect urine specimens.A basal urine specimen will be obtained and volume will be measured. One hour later, 100 and 400 ml of water will be provided to the children and adults respectively to drink. Urine will be further collected after 3 hours of pentagastrin administration (i.e. 2 hours after recording of pentagastrin stimulated tissue resistivity by APT).

Urinary acid (or base) content will be determined using the method of Jorgensen (Varley H, 1980). A measured volume of urine will be acidified with a known volume of hydrochloric acid to liberate carbon dioxide from any bicarbonate present and to dissolve any deposit of phosphate. The mixture will then be boiled to drive off the carbon dioxide and after dilution will be titrated to pH 7.4 with NaoH (0.025 M) in the presence of formaldehyde. Creatinine concentration of urine will be determined using the modified Jaffe reaction (Roche diagnostics) on a COBAS BIO centrifugal fast analyser. This determination will be done within two hours of voiding of urine to prevent change in titratable acidity secondary to evaporation of carbon dioxide from the sample.

6.4.3.3 Determination of Urine acid output : Urinary acid output will be expressed as the urinary H+/creatinine molar ratio for each sample tested (Thomas et al, 1993).

6.5. Risks of the procedures

Pentagastrin test

The gastric intubation, like any clinical procedure is hazardous and involve potential discomfort to the subjects. There have been isolated

instances of arythmia in healthy man. However, we will monitor pulse rate during the procedure to avert such possibilities.

Applied Potential Tomography

Discomfort- nil to slight (putting electrodes around the abdomen may cause slight discomfort to the subjects)

Risk-nil

Measurement of Titratable Acid output in urine:

Discomfort- nil
Risk-nil

5.1 Rationale: Pentagastrin as a gastric acid stimulant.

We have preferred pentagastrin as a gastric - stimulant on consideration of safety, comfort, availability and low cost. Moreover, Peak Acid Output (PAO) occurs within 30 minutes of administration at a dose of 6 µg/kg. Compared with histamine and histalog the side-effects (transient hypotension, tachycardia) of pentagastrin are minor (Baron 1978).

Sample size calculation for children

To determine a sample size, a value of the standard error of the parameter that will be estimated is essential and the following two points need to

(ii) the confidence limits desired for the estimate.
Once these points are finalized, the following equation may be used for calculating the sample size:

$$Z * S.E. = E.$$

For 95% confidence limits, Z = 1.96, and the standard error of a binomial parameter P is $[(P * (1 - P)/ n]^{\frac{1}{2}}$.

In this particular example, if we assume that sensitivity of the test is 80% and allowable error in the estimate is 10%, the S.E will be $[0.16 / n]^{\frac{1}{2}}$. The required sample size will be:

$$1.96 * [0.16 / n]^{\frac{1}{2}} = 0.10$$
$$n = 62.$$

Expecting 10% drop out, the final sample size is rounded up to 70.

Data Analysis

The results will be analysed in two ways: firstly in terms of overall correlation between Gastric Acid Output (GAO) as assessed by Pentagastrin

Test (PGT) with Urinary Acid Output (UAO) and change of gastric resitivity in APT. Secondly, in terms of sensitivity and specificity of UAO and gastric resistivity change as a measure of gastric acid output when compared with PGT. The cut off points of UAO and gastric resistivity changes will be determined by constructing a receiver operator characteristic (ROC) curve in both cases.

9. Rationale

Gastric acid secretion has been linked to increased susceptibility to infectious diarrhoea in adults. No study has been conducted so far to see the role of gastric acid as a risk factor for children developing diarrhoea. The present study will attempt to develop non-invasive tests; to assess gastric acid output in children. If a simple and non-invasive test for gastric acid secretion is available, it would be more feasible to study the relation of low gastric acid secretion to acute and persistent diarrhoea in children.

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Budget

Professional Scientific Staff

Sl.	Details	No	% time	# months	U.S.$	
1.	Dr. S.A. Sarker		20%	12	2,500	
2.	Dr. P.K. Bardhan		10%	12	1,250	
3.	Dr. D. Mahalanabis				1,000	
4.	Research Officer (Lab)		20%	12	400	

Technical Staff

1.	Research Physician	1	100%	12	2,000	
2.	Female Volunteer	2	100%	12	600	
3.	Secretarial service	1	25%	12	400	
4.	Res Fellow (M.Phil student, Dhaka University)	1	100%	12	500	

Travel: International 2,000

Supplies and Materials Medical supply, Office supply, Equipment, lab supply		1,000
Biochemical tests: Titration gastric acid (6x50)=250 Titration urinary acid (2x50)=100	 300 x 10 100 x 10	 3,000 1,000
Micro-Computer Printing and publications Communications Telex, Fax Papers and stationery	1 x $ 2,000 $ 800 $ 500 $ 500 $ 500	2,000 800 500 500 500 --- 4,300
Patient Hospitalisation 80 patients x 2 days x $25/day		4,000
Consultant's Honorarium		2,000
	Total	25,950

CONSENT FORM

(Will be read and explained clearly before consent is obtained)

International Center for Diarrhoeal Diseases Research is planning to undertake a study in the clinical Research Center, Dhaka to develop simple tests to assess acid output from the stomach in children.

We beleive , the new tests will help to undestand the relationship between the diarrhoea and acid output from the stomach. We request you to allow your child to participate in this study. if you agree, the following procedures will be followed:

1. Your child will will be examined thoroughly and will be provided routine care.

2. We will perform follwing tests in two different days.

i) Pentagastrin test

A narrow and soft rubber tube will be swallowed by your child after a fasting period of 4 hours. Fluid from the stomach will be collected through the tube for half an hour. After half an hour of fluid collection Pentagastrin, a medicine which stimulates the stomach to produce acid will be injected at a dose of 6 ug/kg body weight. After 10 minutes of injection fluid will be again collected for anothe period of 1 hour.. The fluid collected will be analysed for acid concentration. Intravenous fluid containing 10% dextrose will be given to maintain your child's energy during the procedure.

ii) Applied Potential Tomography and Measurement of Titratable Acid Output in Urine

These two tests will be performed simultaneously in the other day.
Fasting urine will be collected in the morning. After collection of fasting urine, 16 electroden will be placed in a circle around the upper abdomen. Tomographic images of tissue relativity of the stomach content will be obtained for 30 minutes by Appled Potential Tomography. After 30 minutes pentagastrin will be injected and tomographic images will be obtained again for another 1 hour. Urine will also be collected during this period and will be analysed for titratable acid.

3. The study involves no risk. We will maintain the confedentiality of the results. If you want to know the results of the tests, we shall be happy to let you know as soon as they are available.

4. At any stage of the study, you may withdraw your child from the study, but his/her routine treatment by us will not be hampered. If you have any question to ask, we shall be happy to answer them.

5. If you agree to participate in this study, please sign below.

Signature of the investigator	Signature of witness	Signature or left thump impression of the legal guardian

CONSENT FORM (FOR ADULT VOLUNTEERS)

ll be read and explained clearly before consent is obtained)

ernational Center for Diarrhoeal Diseases Research is planning to undertake a study in the nical Researh Center, Dhaka to develop simple tests to assess acid output from the stomach.

beleive , the new tests will help to undestand the relationship between the diarrhoea and acid put from the stomach. We request you to participate in this study. if you agree, the following cedures will be followed:

You will be examined thoroughly and will be provided routine care.

We will perform follwing tests in two different days.

Pentagastrin test

arrow and soft rubber tube will be swallowed by you and will be introduced into the stomach after overnight fast. Fluid from the stomach will be collected through the tube for half an hour. After f an hour of fluid collection Pentagastrin, a medicine which stimulates the stomach cells to duce acid will be injected at a dose of 6 ug/kg body weight. After 10 minutes of injection fluid l be again collected for another period of 1 hour. The fluid collected will be analysed for acid centration.

Applied Potential Tomography and Measurement of Titratable Acid Output in Urine

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The study involves no risk. We will maintain the confidentiality of the results. If you want to w the results of the tests, we shall be happy to let you know as soon as they are available.

At any stage of the study, you may withdraw your name from the study, but the routine treatment us will not be hampered. If you have any question to ask, we shall be happy to answer them.

If you agree to participate in this study, please sign below.

nature of the estigator	Signature of witness	Signature or left thump impression of the Volunteer

সম্মতি পত্র (প্রাপ্ত বয়স্কদের জন্য)

(সম্মতি নেয়ার পূর্বে এপত্রটি ভাল করে পড়ে এবং বুঝিয়ে দেয়া হবে)

আন্তর্জাতিক উদরাময় : গবেষনা কেন্দ্রস্থ ক্লিনিক্যাল রেসার্চ সেকশন

আপনার পাকস্থলি নিঃসৃত এসিড পরিমাপের জন্যে একটি সহজ পদ্ধতি উদ্ভাবনের গবেষনার পরিকল্পনা করতে যাচ্ছে।

আমরা বিশ্বাস করি, এই নতুন পদ্ধতি পাকস্থলি নিঃসৃত এসিড এবং ডায়রিয়া রোগের বিদ্যমান সম্পর্ক সম্পর্কে জানতে অধিকতর সহায়তা করবে। আমরা আপনাকে এ গবেষনায় অংশগ্রহনের জন্যে আমন্ত্রণ করছি।

আপনি যদি রাজী থাকেন, তবে নিম্নোক্ত বিষয়গুলো অনুসরণ করা হবে।

১। আপনাকে ভালভাবে পরীক্ষা করা হবে এবং হাসপাতালে প্রচলিত চিকিৎসাদি প্রদান করা হবে।

২। আমরা নিম্নোক্ত পরীক্ষাগুলো বিভিন্ন দিনে করবো :

পেন্টাগেস্ট্রিন পরীক্ষা

সকালবেলা খালি পেটে আসার পর একটি সরু নলবিশিষ্ট রাবারের নল আপনাকে গিলতে দেয়া হবে এবং পাকস্থলি পর্যন্ত প্রবেশ করানো হবে। এই সরু নলের সাহায্যে পাকস্থলির রস আধঘন্টা ধরে সংগ্রহ করা হবে। এরপর পেন্টাগেস্ট্রিন (যে ঔষধ পাকস্থলির কোষকে উত্তেজিত করে এসিড নিঃসরণ করে) ৬ মাইক্রোগ্রাম/প্রতি কি: গ্রাম শরীরের ওজন অনুপাতে ইঞ্জেকশন দেয়া হবে। ইঞ্জেকশনের ১০মিনিট পর আবার ১ঘন্টা পর্যন্ত পাকস্থলির রস সংগ্রহ করা হবে। এই সংগৃহীত রস থেকে পরে এসিড পরিমাপ করা হবে।

এপ্লায়ড পোটেনশিয়াল টোমোগ্রাফি এবং গ্রসারে এসিড বিশ্লেষণ

এই দুটি পরীক্ষা অন্য একদিন একসাথে করা হবে। সকালে খাওয়ার পূর্বে আপনার শ্বাসনল সংগ্রহ করা হবে। শ্বাসনল সংগ্রহের পর ১৬টি ইলেকট্রোড আপনার পেটের উপরি অংশে চুক্তাকারে লাগানো হবে। এপ্লায়ড পোটেনশিয়াল টোমোগ্রাফি মেশিনের সাহায্যে পাকস্থলিস্থ উপাদানের পরিবাহীতা বা প্রতিরোধকতা ৩০ মিনিট ধরে নির্ণয় করা হবে। এরপর পেন্টাগেস্ট্রিন (যা আগে উল্লেখ করা হয়েছে) একই ডোজে ইঞ্জেকশন দেয়া হবে এবং ইঞ্জেকশনের ১০মিনিট পর ১ঘন্টা ধরে পাকস্থলিস্থ উপাদানের পরিবাহীতা বা প্রতিরোধকতা দ্বিতীয়বার নির্ণয় করা হবে। আপনার হতে সর্বমোট ৩০মিলি রক্ত এসময়ে এসিড পরিমাপের জন্য সংগ্রহ করা হবে।

৩। এই গবেষনায় কোন বিপদ বা আশংকা নেই। আমরা পরীক্ষার ফলাফল গোপন রাখবো। আপনি যদি পরীক্ষার ফলাফল জানতে আগ্রহী থাকেন তবে আমরা তা জানাবো।

৪। গবেষনার যে কোন পর্যায়ে আপনি গবেষনা থেকে নাম প্রত্যাহার করিতে পারেন। এতে আপনার প্রচলিত চিকিৎসার কোনরূপ ক্ষতি হবে না। আপনার যদি কোন প্রশ্ন থাকে তবে আমরা আনন্দের উত্তর প্রদান করব।

৫। আপনি যদি এই গবেষনায় অংশগ্রহনে রাজী থাকেন তবে নীচে স্বাক্ষর প্রদান করুন।

গবেষকের স্বাক্ষর _____ স্বাক্ষীর স্বাক্ষর _____ অভিভাবকের স্বাক্ষর / ছিপ সহি _____

CONSENT FORM (FOR CHILDREN)

Will be read and explained clearly before consent is obtained)

nternational Center for Diarrhoeal Diseases Research is planning to undertake a study in the linical Researh Center, Dhaka to develop simple tests to assess acid output from the stomach in hildren.

e beleive , the new tests will help to undestand the relationship between the diarrhoea and acid utput from the stomach. We request you to allow your child to participate in this study. if you gree, the following procedures will be followed:

. Your child will will be examined thoroughly and will be provided routine care.

. We will perform follwing tests in two different days.

) Pentagastrin test

narrow and soft rubber tube will be swallowed by your child and will be introduced in the stomach fter a fasting period of 4 hours. Fluid from the stomach will be collected through the tube for alf an hour. After half an hour of fluid collection Pentagastrin, a medicine which stimulates the tomach to produce acid will be injected at a dose of 6 ug/kg body weight. After 10 minutes of njection fluid will be again collected for anothe period of 1 hour.. The fluid collected will be nalysed for acid concentration. Intravenous fluid containing 10% dextrose will be given to maintain our child's energy during the procedure.

ii) Applied Potential Tomography and Measurement of Titratable Acid Output in Urine

hese two tests will be performed simultaneously in the other day.
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. The study involves no risk. We will maintain the confidentiality of the results. If you want to now the results of the tests, we shall be happy to let you know as soon as they are available.

. At any stage of the study, you may withdraw your child from the study, but his/her routine reatment by us will not be hampered. If you have any question to ask, we shall be happy to answer hem.

. If you agree to participate in this study, please sign below.

ignature of the investigator	Signature of witness	Signature or left thump impression of the legal guardian

সম্মতি পত্র (শিশুদের জন্য)

(সম্মতি নেয়ার পূর্বে এ পত্রটি ভালো করে পড়ে ও বুঝিয়ে দেয়া হবে)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্রস্থ ক্লিনিক্যাল রিসার্চ সেক্টর। আপনার সন্তানের পাকস্থলি নিঃসৃত এসিড পরিমাপের জন্য একটি সহজ পদ্ধতি উদ্ভাবনের গবেষণার পরিকল্পনা করতে যাচ্ছে।

আমরা বিশ্বাস করি, এই নতুন পদ্ধতি পাকস্থলি নিঃসৃত এসিড এবং ডায়ুরিয়া রোগের বিদ্যমান সম্পর্ক সম্বন্ধে জানতে অধিকতর সহায়তা করবে। আমরা আপনাকে অনুরোধ করছি আপনার সন্তানকে এই গবেষণায় অংশগ্রহণে অনুমতি দেয়ার জন্য।

আপনি সম্মতি প্রদানে রাজী থাকলে, তবে নিম্নোক্ত বিষয়গুলো অনুসরণ করা হবে:

১। আপনার সন্তানকে ভালভাবে পরীক্ষা করা হবে এবং হাসপাতালে প্রচলিত চিকিৎসাদি প্রদান করা হবে।

২। আমরা নিম্নোক্ত পরীক্ষাগুলো বিনা মূল্যে করবো:

পেন্টাগাস্ট্রিন পরীক্ষা

কিছুক্ষন (৪ ঘন্টা) না খেয়ে রাখার পর একটি অতি সরু নরম রাবারের নল আপনার সন্তানকে গিলতে দেয়া হবে এবং পাকস্থলি পর্যন্ত প্রবেশ করানো হবে। এই নল মারফত পাকস্থলির রস আধঘন্টা ধরে সংগ্রহ করা হবে। এরপর পেন্টাগাস্ট্রিন (যে ঔষধ পাকস্থলির কোষকে উত্তেজিত করে এসিড নিঃসরণ করে) ও মাইক্রোগ্রাম/প্রতি কি: গ্রাম ওজন অনুপাতে ইঞ্জেকশন দেয়া হবে। ইঞ্জেকশনের ১০ মিনিট পর আরও ১ ঘন্টা পর্যন্ত পাকস্থলির রস সংগ্রহ করা হবে। এই সংগৃহীত রস থেকে পরে এসিড পরিমাপ করা হবে। আপনার সন্তানের চাহিদা মোতাবেক জন্য পরীক্ষা চলাকালীন তাদের যুক্তিসঙ্গত সান্নিধ্য প্রদান করা হবে।

এপ্লায়েড পোটেন্সিয়াল টেলেমেট্রাফি এবং প্রসাবে এসিড নির্ণয়

এই দুটি পরীক্ষা অন্য একদিন একসঙ্গে করা হবে। সকালে যাওয়ার পূর্বে আপনার সন্তানের প্রস্রাব সংগ্রহ করা হবে। প্রস্রাব সংগ্রহের পর ১৬ টি ইলেকট্রোড আপনার সন্তানের পেটের উপরি অংশে বৃত্তাকারে লাগানো হবে। এপ্লায়েড পোটেন্সিয়াল টেলেমেট্রাফি মেশিনের সাহায্যে পাকস্থলিস্থ উপাদানের পরিবাহীতা বা প্রতিদ্বন্দ্বকতা ৩০ মিনিট ধরে নির্ণয় করা হবে। এরপর পেন্টাগাস্ট্রিন (যা আগে উল্লেখ করা হয়েছে) একই ডোজে ইঞ্জেকশন দেয়া হবে এবং ইঞ্জেকশনের ১০মিনিট পর ১ঘন্টা ধরে পাকস্থলিস্থ উপাদানের পরিবাহীতা বা প্রতিদ্বন্দ্বকতা পুনরায় নির্ণয় করা হবে। আপনার সন্তানের প্রস্রাবও একসাথে এসিড পরিমাপের জন্য সংগ্রহ করা হবে।

৩। এই গবেষণায় কোন বিপদ বা অসুবিধা নেই। আমরা পরীক্ষার ফলাফল গোপন রাখবো। আপনি যদি পরীক্ষার ফলাফল জানতে আগ্রহী থাকেন তবে আমরা তা জানাবো।

৪। গবেষণার যে কোন পর্যায়ে আপনার শিশুকে গবেষণা থেকে প্রত্যাহার করিয়ে নিতে পারেন। এতে আপনার সন্তানের প্রচলিত চিকিৎসার কোনরূপ ত্রুটি হবে না। আপনার যদি কোন প্রশ্ন থাকে তবে আমরা সানন্দে উত্তর প্রদান করব।

৫। আপনি যদি এই গবেষণায় অংশগ্রহণে রাজী থাকেন তবে নীচে স্বাক্ষর প্রদান করুন।

গবেষকের স্বাক্ষর _____ অভিভাবকের স্বাক্ষর / টিপ সই _____