V2

Revised

Date

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. M. Huq

Application No. 83-017(P)

Title of Study Presence and biochemical basis of an increased serum anion gap in the metabolic acidosis of cholera

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

Project status:
(X) 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 NA
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.

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

Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

(PILOT STUDY)

1. Title: Presence and biochemical basis of an increased serum anion gap in the metabolic acidosis of cholera

2. Investigators: M. Huq and A. Waheed

   Consultants: T. Butler and R. Islam

3. Starting Date: September 1983

4. Completion Date: December 1983

5. Total Direct Cost: $2,297

6. Scientific Program Head: Thomas Butler, M.D.

This protocol has been approved by the Pathogenesis-Therapy Working Group.

Signature of Scientific Program Head: Thomas Butler

Date: July 21, 1983

7. Abstract Summary:

Twenty adult patients with cholera will be studied to define the presence, and biochemical basis, of an increased serum anion gap in the metabolic acidosis of cholera. Blood will be obtained for biochemical determinations on admission and at convalescence prior to

release. Acute and convalescent anion gaps will be calculated, and the charge, DAG, explained on the basis of changes in the ionic equivalence of the normal ionic constituents of blood, and/or addition of organic acids such as lactate or keto anions.

8. Reveiws:

a. Research Involving Human Subjects: _____

b. Research Review Committee: _____

c. Director: _____

SECTION II - RESEARCH PLAN

Presence and Biochemical Basis of an Increased Serum Anion Gap in the metabolic acidosis of Cholera.

A. Introduction:

1. Objective:

Metabolic acidosis can be divided into two types: a hyperchloremic non-anion gap and a normochloremic anion gap variety. Diarrhea, with loss of bicarbonate in stool, results in the former. Cholera appears to be an exception in that it results in an anion gap acidosis. This study intends to evaluate the characteristics of the metabolic acidosis of cholera. The presence of an anion gap will be prospectively documented and its biochemical basis defined.

2. Background:

Metabolic acidosis and dehydration are the clinical hallmarks of cholera, which if incorrected, lead rapidly to death. The pathophysiology of saline depletion and dehydration in cholera, and their treatment, have been well described[1]. The metabolic acidosis has been less well studied, particularly its biochemical characterization, although its treatment[2] controversies in treatment[3], and complications of treatment[4], as well as its respiratory and hemodynamic effect[5] have received attention.

The plasma and stool electrolyte compositions in cholera have been previously elucidated.[6,7,8]. The metabolic acidosis has been

attributed to bicarbonate loss in the diarrheal stool. As such, this should result in a non-anion gap hyperholeremic metabolic acidosis[9]. There is evidence in the literature, culled by arithematic manipulation of the reported serum electrolytes, that an increased anion gap is present in cholera patients. This observation is confirmed from retrospective data from ICDDR,B[10].

The serum anion gap is expressed as the difference between normally measured cations and anions in Meq/L[9,11] that is AG=Na - (Cl + HCO_3), or numerically, 10 - 140 - (105+25). Since electrical neutrality is m maintained in serum, the difference between the unmeasured anions and cations must also equal the anion gap[11]. The unmeasured anions consist of protein, inorganic phosphate and sulfate, and organic acids; and represent (4 6) 14, 2, 1 and 5 MEq/L, respectively[9,11]. The unmeasured cations include potassium, calcium and magnesium. They represent (4.5) (1.5) 5, 5, and 2 mEq/L, respectively[9,11].

The diagnosis of metabolic acidosis is based on a low arterial blood pH and a low serum biocarbonate concentration. If there is a compensatory increase in the serum chloride concentration, an increased serum anion gap does not result. This is true of the metabolic acidosis resulting from diarrhea, an obstructed uretero-ileostomy or uretero sigmoidostomy, renal tubular acidosis, and treatment with a carbonic anhydrase inhibitor or ammonium chloride[9]. When there is no compensatory hyperchloremia, an increased serum anion gap occurs. The most common explanation being addition of measured organic acids such as occurs in lactic or ketoacidosis, or with ingestion of salicylates, methanol, gluteraldehyde paraldetyde or ethylene glycol[9]. Other equally

tenable, although less common, explanations for the increase in the serum anion gap include a decrease in the normally unmeasured cations, or an increase in the unmeasured anions. The increase in inorganic phosphates and sulfates which occurs in chronic renal failure is an example of this latter mechanism for the increased serum anion gap.

The water and saline depletion of cholera leads to clinicallyy varying degrees of dehydration and ultimately shock. The latter may result in lactic acidosis. Dehydration can result in a slight, but reportedly imperceptible, increase in the anion gap[9]. Although theoratically, marked dehydration can lead to the concentration of the normally present impereable anionic constituents of plasma, most importantly protein, and can therefore result in an increased anionic equivalence[12] and anion gap. This posibility, has not been reported to be of clinical significance, or described in detail. A recent study[13] to define the biochemical basis of the increased increment in the anion gap in hospitalized patients with metabolic acidosis in the United States concluded that 62% of the incremental anion gap could be accounted for by lactate and ketoanions. Only 15% was attributable to changes in the ionic equivalents of the normally present anionic and cation constituents of serum. A significant percentage of the incremental anion gap remained unexplained, despite sophisticated biochemical determinations.

Although changes in serum protein[14,15], and magnesium[16] have been

reported in cholera, the existence of an anion gap metabolic acidosis in cholera has not received attention to date, nor has its biochemical basis been investigated.

B. SPECIFIC AIMS:

1. To measure acute and convalescent serum electrolytes, protein, trace minerals, organic acids, and blood pH in patients with cholera.

2. To calculate the acute and convalescent anion gap and the change in anion gap, DAG.

3. To define the biochemical basis of DAG, by evaluating for the addition of organic anions, or the change in normally 'unmeasured' serum anions and cations.

C. METHODS OF PROCEDURE:

1. Patient Selection - Twenty male patients age 18 years and above who present to ICDDR,B with watery diarrhea will be considered. To be selected they should have the following criteria:
   a. stool should be positive for vibrio by dark field and confirmed by culture
   b. patient should have severe dehydration
   c. patient should not have any preexisting renal disease, diabetics, recent history of ingestion of salicylate or on treatment with a carbonic anhydrase inhibitor.
2. Informed consent - Each patient will be explained about the study. They will be asked to give permission to obtain 17cc blood on admission and at convalescence prior to release.

3. Rhydration - All patients will be rehydrated with acetate solution to meet their deficit. Any further losses in the stool will be replenished adequately. Capsule tetracycline 500mg/6 hr for 2 days will be given to all patients..

4. Serum biochemical measurements - 17cc of venous blood will be obtained by routine sterile technique at admission before administration of any fluid and at convalescence prior to release. Sodium, potassium, chloride and total CO_2 will be measured in mEq/L and calcium, magnesium and inorganic phosphate and sulfate in mg/k. Serum will also be qualitatively assayed for acetoacetate by ACETEST reagent. Although arterial blood is supperior for lactate and pH determinations, each can be adequately determined on venous blood. The quantitative determination of lactate with LDH, GPF and NAO will be performed using a "Monotest Lactatekit (Boehringer-Mannhem Co.West Germany) following the technique of "Noel" within 2 hr of specimen collection.

5. Calculation of serum ionic equivalence - Calcium and magnesium ionic equivalence can be calculated from the following formulas:

$$\text{Ca mEq/L} = \frac{\text{Ca mg/dl} \times 10}{20\ (\text{½ at. wt})} \qquad \text{mg mEq/L} = \frac{\text{mg mg/dl} \times 10}{12\ (\text{½ at wt})}$$

The ionic equivalence of protein and inorganic phosphate can be calculated from the following formulas:

$$\text{Negative charge in mEq/L} = 10.3\ (\text{pH}-5.66) \times 0.1\ (\text{Total protein in gm/dl}) \quad (13)$$

$$\text{Phosphate in mEq/L} = C\left(10^{[\text{p in mgm/dl}]}/31\right) \quad (13)$$

$$C = 1+\left[10^{\text{pH}-6.8}\left(10^{\text{pH}-6.8}+1\right)\right]$$

Lactate in mg/dl can be converted to mEq/L by dividing the concentration by 0.9.

6. Serum from selected patients will be subjected to millipore filter centrifugation to remove serum proteins. The anion gap will be calculated before centrifugation, and on the filtrate. The percentage reduction in anion gap will be measured and compared to the calculated value of protein contribution by the direct measurements of ions and proteins.

D. SIGNIFICANCE AND RATIONALE

Cholera constitutes a serious health problem in developing countries such as Bangladesh. Since the demonstration in the early 1960's of solute coupled active intestinal sodium transport, application of this information to the rational therapy of cholera has resulted in a marked decrease in mortality. Modifications of the constituents of both oral and intravenous rehydration solutions are being continuously investigated. Such modification requires an understanding of the biochemical and physiologic perturbations of cholera. This study proposes to define the biochemical basis of the anion gap metabolic acidosis of cholera. Such a demonstration would also contribute an important addition to the acid-base literature, especially if a large percentage of the incremental anion gap is shown to be secondary to charges in ionic equivalence, particularly serum anionic protein.

E. FACILITIES REQUIRED:

The present Hospital and Biochemistry Laboratory Facilities are sufficient to carry out this study.

F. ANALYSIS OF DATA

Data sheets will be kept for every patient listing all quantitative and qualitative acute and convalescent biochemical determinations. The anion gap, and DAG, will be calculated. The contribution to DAG of changes in acute and convalescent ionic equivalence will be expressed as percentages.

REFERENCES:

1. Carpenter, CCJ. Clinical and pathophysiologic features of Diarrhea caused by vibrio cholera and Escherichia coli. In Secretory Diarrhea. Ed. M. Field, J. Fordtron and S. Schultz. American Physiological Society, Bethesda, Maryland, 1980

2. Carpenter, CCJ. Oral Rehydration. Is it as good as parenteral therapy. (Editorial) New Eng. J. Med. 306:1103, 1982

3. Greenough, WB. Oral replacement therapy in diarrhea: Consensus and contraversy. In Secretory Diarrhea. Ed. m. Field, J. Fordtran, and S. Schultz. American Physiological Society. Bethesda, Maryland, 1980

4. Greenough, WB. et al. Pulmonary oedema associated with acidosis in patients with cholera. Trop. Geogr. Med. 28:86-90, 1976

5. Harvey, RM et al. Hemodynamic studies on cholera of hypovolemia and acidosis. Circulation. 37:709-728, 1968

6. Watten, RH et al. Water and electrolyte studies in cholera. J. Clin. Invest. 38:1879, 1959

7. Pierce, NF et al. Replacement of water and electrolyte losses in cholera by an oral glucose-electrolyte solution. Ann. Intern. Med. 70:1173, 1969

8. Carpenter, CCJ et al. Clinical studies in Asiatic cholera. Parts I-VI. Bull. Johns. Hopkins Hospital. 118:165, 1966

9. Emmet, M, Narins, RG. Clinical use of the anion gap. Medicine (Baltimore) 56:38, 1977

10. Islam, MR et al. Labon-Gur (common salt and brown sugar) oral rehydration solution in the treatment of diarrhea in adults. J. Trop. Med. Hyg. 83:415, 1980

11. Oh MS, Carrol HJ. The anion gap New Engl. J. Med. 297:814, 1977

12. Madias, NE et al. Increased anion gap in metabolic alkalosis: the role of plasma-protein equivalency. New Engl. J. Med. 300:1421, 1979

13. Gabow, PA, et al. Diagnostic importance of an increased serum anion gap. New Engl. J. Med. 303:854, 1980

14. Chakrabarty, AS. et al. Serum iron and serum proteins in some infections diseases. Am J. Med. Sci. 250:667, 1965

15. Panse MV et al. Serum protein changes by paper electrophoresis in rabbit and human cholera. Indian J. Path. Bact. 8:138, 1965

16. Dutta, JK et al. Serum magnesium study in cholera and non choleric gastroenteritis. Trans. R. Soc. Trop. Med. Hyg. 71:263, 1977

17. Kreisberg, RA. Lactate homeostasis and lactic acidosis. Annals Int. Med. 92:227, 1980

18. Van Leeuwen, AM. Net cation equivalency ('base binding power') of the plasma proteins : a study of ion-protein interaction in human plasma by means of in vivo ultrafiltration and equilibrium dialysis. Acta. Med. Scand. (suppl) 422:1-212, 1964

19. Oliva, PB. Lactic Acidosis, Am. J. Med. 48:209, 1970

20. Noll, F. Lactate Determination with LDH, GPT and NAD. In. Methods of Enzymatic analysis. Volume 3. Ed. by H.U. Bergmeyer, Academic Press Inc., New York. p. 1475, 1983

21. **Controni, G., Rodriguez, W.J., Hicks, J.M. et al: Cerebrospinal fluid lactic acid levels in meningitis. J.Pediatrics. 91:379,1977**

22. Rosenberg, JC, Rush BF. An enzymatic-spectrophotometric determination of pyruvic and lactic acid in blood. Clin. Chem. 12:299, 1966

ABSTRACT SUMMARY:

1. This study proposes to define the presence and biochemical basis of the increased serum anion gap metabolic acidosis in cholera.

2. The risks are negligable, and include only those related to blood drawing. Only previously healthy adult males will be selected in order to avoid risk to anemic or pregnant persons.

3. Risks will be minimized by adhereing to the usual sterile technique of blood drawing.

4. Confidentiality will be maintained by use of patient hospital number rather than name.

5. Signed informed consent will be obtained.

6. There will be no interview.

7. The potential benefits include, for the patient, the usual careful treatment that all patients at ICDDR,B receive, and for society, the elucidation of the biochemical basis of the increased serum anion gap acidosis of cholera, which may further benefit the ongoing modification of existing rehydration solutions.

8. This study requires only the use of blood from patients with cholera.

SECTION III - BUDGET

(DETAILED BUDGET)

1. Personnel Services

Name	Postition	Period	%Effort	Taka	Dollar
Dr. Mahfuzul Huq	Pr. Investigator	4 mths	30	-	145
Mr. M.A. Wahed	"	"	20	-	180
Dr. R. Islam	Consultant	"	-	-	-
Dr. T. Butler	"	"	-	-	-
Not named	Biochemistry Technician	"	30	-	193

2. Supplies and materials:

Stool cultures	50 x Tk.5 = 250	10
Blood electrolytes	50 x $2.02 per test	101
Stool electrolytes	50 x $2.02 per test	101
Blood protein	50 x $1.80 per test	90
Stool protein	50 x $1.80 per test	90
Monotest lactate (estimated)		500
Blood clacium. Magnesium	50 x $2.74 per test x 2	274
Syringes, needles	50 x $1	50

3. Equipment - 0

4. Patient hospitalization - 150 Tk. per day for 30 patients 3 days each = 9000 — 563

5. Outpatient care - 0 — -

6. ICDDR,B transport - 0 — -

7. Travel and transportation - 0 — -

8. Rent - 0 — -

9. Printing - 0 — -

10. Other contractual services - 0 — -

11. Construction/Renovation - 0 — -

$2,297

BUDGET SUMMARY

		US$
1.	Personnel services	518.00
2.	Supplies & Materials	1,216.00
3.	Equipment	-
4.	Patient hospitalization	563.00
5.	Outpatient care	-
6.	ICDDR,B transport	-
7.	Travel & transportation	-
8.	Rent	-
9.	Printing	-
10.	Other contractual services	-
11.	Construction/Renovation	-
	Total :	2,297.00

(Conversion rate $1 = Tk.24)

ICDDR,B (Anion gap)

CONSENT FORM

ICDDR,B is an international centre for research on diarrheal disease. You have become ill with cholera and will be treated routinely with fluids and antibiotics. The Centre wishes to draw blood (exactly 17cc) to measure minerals and acids and protein in your blood. The same amount will be drawn 3 days later before you leave the hospital. The results will not change your treatment but will permit us to learn more about cholera. The amount of blood we take is not at all dangerous to you during this illness.

If you do not wish to participate in this study, your treatment will be the same.

If you agree to participate, please sign here.

_____

Patient

_____

Investigator

_____

Date

আন্তর্জাতিক ঔদরাময় গবেষনা কেন্দ্র, বাংলাদেশ

সম্মতি পত্র

আই, সি, ডি, ডি, আর, বি - একটি গবেষনা কেন্দ্র। আপনি ঔদরাময় রোগে আক্রান্ত হয়ে এখানে চিকিৎসার জন্য এসেছেন। এখানে আপনাকে নিয়মিত ভাবে স্যালাইন ও এন্টিবায়োটিক দিয়ে চিকিৎসা করা হবে। আপনার রক্তের অম্লত্ব, ক্ষারত্ব পদার্থ ও অন্যান্য জরুরী পদার্থ পরীক্ষা করার জন্য ভর্তির সময় আপনার শিরা থেকে ২৭ সিসি রক্ত নেওয়া হবে। তিন দিন পরে আবার হাসপাতাল ছাড়ার আগের দিন সমপরিমান রক্ত নেওয়া হবে। এই সব পরীক্ষার ফলাফলের জন্য আপনার চিকিৎসার কোন তারতম্য ঘটবে না বরং এই ফলাফল ঔদরাময় রোগীকে সুস্থ্য করে তোলার ব্যাপারে আমাদের নতুন জ্ঞান দেবে। যে পরিমান রক্ত আমরা সংগ্রহ করবো তাতে আপনার কোন প্রকার ক্ষতি হবে না।

যদি আপনি এ গবেষনায় অংশ গ্রহন করতে না চান তাতেও আপনার সুচিকিৎসার কোন ব্যঘাত ঘটবে না।

আপনি যদি অংশ গ্রহনে ইচ্ছুক হোন তবে নিম্নে সই করুন।

রোগীর স্বাক্ষর।

তারিখ

গবেষকের স্বাক্ষর।