29

Date 27.1.85

ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigator Dr.D. Patte

ication No. 85-006

e of Study "Hyponatremia in Shigella Infections"

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B) Ford Found

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

e the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects Yes No
(c) Minors or persons under guardianship (Yes) No

oes 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)

es the study involve:
) Use of records, (hospital, medical, death, birth or other) (Yes) No
) Use of fetal tissue or abortus Yes (No)
) Use of organs or body fluids (Yes) No

e subjects clearly informed about:
) Nature and purposes of study (Yes) No
) Procedures to be followed including alternatives used (Yes) No
) Physical risks Yes No
) Sensitive questions Yes No
) Benefits to be derived Yes No
) Right to refuse to participate or to withdraw from study (Yes) No
) Confidential handling of data (Yes) No
) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No

NA

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
   NA Questionnaire or interview schedule *

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

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

Principal Investigator

Trainee

85-506
28/1/85

SECTION I - RESEARCH PROTOCOL

1. Title : Hyponatremia in Shigella infections

2. Investigators : D. PATTE Principal investigator

Physician to be named, Co-investigator

3. Starting date : March 1985

4. Completion date : March 1986

5. Total direct cost : 5467 US$

6. Scientific program head : Thomas BUTLER

This protocol has been approved by the Pathogenesis & Therapy working group.

Signature of scientific program head : Jean Cifuentes

Date : 28/1/85

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ABSTRACT SUMMARY

The occurence of severe hyponatremia during Shigella infections is a well known phenomenon, but it has not been studied per se and its mechanisms are still unknown.

Evidence suggests a centrally mediated mechanism which might be related to the presence of circulating toxin :

1. It seems unlikely that sodium depletion could be the only cause of severe hyponatremia. Even in the most serious cases of diarrhea, stool sodium loss is not severe enough to cause hyponatremia. In addition, the kidney should normally be able to conserve sodium and compensate partially for the fall in sodium due to stool losses.

2. No explanation has been given to the fact that hyponatremia is more frequent and more severe during infections due to shigella dysenteriae type 1 than with other shigella strains. Shigella dys.1 is known to produce toxin in a much larger amount than other strains.

The hypothesis tested in this protocol is that hyponatremia in shigella dysenteriae type 1 infections is caused by an inappropriate secretion of anti-diuretic hormone. This can be tested by studying the kidney's ability to eliminate free water.

It is therefore proposed to investigate patients suffering from shigellosis and to challenge them with a measured amount of plain water to study their dilution capabilities.

This protocol will also require that all patients enrolled in the study have blood drawn to assay ADH and toxin levels.

REVIEWS

Research involving human subjects_____

Research review committee_____

Director_____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective :

The protocol's objective is to elucidate the mechanism of hyponatremia in schigellosis due to shigella dysenteriae type 1 and to test the hypothesis of the presence of an inappropriate secretion of anti-diuretic hormone (SIADH). If SIADH exists in shigellosis, it could be related to the presence of the so-called neurotoxin.

2. Background :

Hyponatremia has been recognized as a synmptom of severe shigellosis when the pathogen is shigella dysenteriae type 1. It is thought to be associated with other complications, such as the HUS, and to be often concommitent with neurological symptoms, mainly stupor/lethargy and convulsions (1). However, little attention has been paid to its pathophysiological mechanisms, and it is still unclear wether neurological symptoms may be due to hyponatremia or are due to the action of the toxin itself.

While conducting a study on Shigella sepsis (with Dr. M. STRUELENS in 1983-84) we reviewed previous cases of sepsis and we also reviewed charts from non-bacteremic patients as a control group.

From these data, we were able to discover significant differences in serum sodium levels in patients with Shigella flex. and Shigella shiga infection.

	BACTEREMIC PATIENTS		NON BACTEREMIC PATIENTS	
	Sh.Shiga	Sh.Flex	Sh.Shiga	Sh.Flex
n=	34	38	23	23
[Na]=	123.08	130.8	128.08	132.17
+/-	8.54	7.33	7.62	7.03
p=	.0001		.049	

Although profound hyponatremia was mainly associated with Shigella shiga species, it has also been observed with other strains which cause hyponatremia ranging from moderate (125-130) to severe (100-125). Hyponatremia was worse in patients with Shigella shiga bacteremia and was less severe in patients with only Shigella shiga diarrhoea (p=.027). This may favour the hypothesis of a toxin mediated mechanism. Another puzzling feature in those patients was the duration of hyponatremia for several days after correction of fluid/electrolytes imbalance, although it was gradually improving.

In fact, apart from inappropriate secretion of ADH, other main mechanisms responsible for hyponatremia have to be ruled out or dealt with in the proposed study :

a. Sodium depletion : Fecal excretion of electrolytes has already been found to be sometimes high in dysentery. However, it is unlikely to be responsible for profound hyponatremia in these patients because they pass relatively small amounts of stool in comparison with those having watery diarrhoeas (2). In addition, the kidney is able to limit the sodium losses almost to a null excretion and should normally prevent hyponatremia in these cases. This hypothesis also doesn't account

for the observed difference in natremia between Shigella shiga cases and other Shigella strains. However, since a long lasting hyponatremia may change both the regulation mechanisms of fluids volume and the intra-cellular potassium content (3,4), patients with a history of diarrhoea for more than four days must be excluded from the study.

b. Appropriate secretion of anti-diuretic hormone : Several physiological or pathological situations provoke ADH secretion normally, such as acute and sustained pain, stress, and hypovolemia (5,6). In this regard, complete rehydration and control of fluid balance must be achieved before a patient can be definitely enrolled in this study.

c. A third condition, namely severe potassium depletion, may decrease to some extent the serum level of sodium according to Edelman's ratio :

$$[Na] = \frac{\text{Na ech. + K ech.}}{\text{Total body water}}$$

where Na ech. = Exchangeable Na
and K ech. = Exchangeable K

Hyponatremia from any cause may lower the circulating level of the ADH (7). Therefore, the presence of any serum ADH under these conditions would be an abnormal finding.

d. Hyponatremia and severe infections. It has long been recognized that hyponatremia may occur in severe infections. This was first studied in acute pneumococcal lobar pneumonia during the 1920's and the 1930's. (8,9). It was further stated that lung diseases,whether acute (10,11)

or chronic, such as tuberculosis (12,13), led to hyponatremia through a possibly ADH mediated mechanism, as well as other infections such as meningitis (14).

Hence, patients with severe infections other than invasive diarrhoea will not be enrolled in the study.

It has also been speculated that in other types of infections, such as peritonitis (15), hyponatremia could be due to exessive production of water under increased metabolism conditions (16,17). However, it remains unclear why water generated in excess is not excreted.

e. Hyponatremia and severe malnutrition. This point is well established and investigated. Therefore, severely malnourished patients (3rd degree) will be excluded.

Assessing an inappropriate secretion of ADH can be achieved through measurement of plasma and urine osmolality and calculating free water and osmolar clearances after a calibrated water load

$$\text{Osm. clearance} = \frac{\text{U.Osm} \times \text{V}}{\text{P.Osm}}$$

where : U.Osm = Urine osmolality
P.Osm = Plasma osmolality
V = Urine flow (ml/min)

and

$$\text{Free Water Clearance} = \text{V} - \text{Osm. Clearance}$$

Under normal circumstances (normovolemic patient) a load of 20ml/kg of plain water after overnight fluid restriction should produce the following : (4)

-60% of the total water load should be evacuated within 2 hours,

-Urine osmolality should drop sharply below the plasma osmolality,

-Free water clearance should turn positive.

Patients with normal kidneys who are unable to respond appropriately as described above to a free water load are suffering from SIADH.

This procedure enables one to indirectly test for the presence of ADH. But it gives no information as how important the secretion is. The only way to answer the question is by measuring the ADH plasma level. Although such an RIA is not currently available, it is wise to save some serum for eventual dosage of the hormone. In the meantime, assaying the level of circulating toxin, either by the means of monoclonal antibodies (18) or indirectly by quantifying the level of circulating anti-toxin antibodies (19,20), could lead to interesting findings with regard to the biological action of this still poorly known toxin.

B. SPECIFIC AIMS

1. To evaluate the presence of circulating ADH in hyponatremic patients infected with Shigella dysenteriae type 1 after rehydration.
2. To compare this group with hyponatremic patients infected with other strains of whigella species under the same conditions, and with isonatremic patients infected with any strain of Shigella.
3. Eventually, to establish correlation between the levels of circulating anti-diuretic hormone d Shigella toxin.

C. METHODS OF PROCEDURE

1. Patients selection

Patients will be selected according to the following criteria :

- Age : above one year
- Sex : both
- Proven Shigellosis lasting for less than 4 days
- No pre-existing renal disease
- No cardiac failure
- No diabetes
- No other infection
- No treatment with a diuretic within the past 24 hours.
- Nutritional status: above 3rd degree (Gomez)

A total of 40 patients will be studied :

- 10 with Shigella dysenteriae 1 infection and hyponatremia
- 10 with other Shigella species and hyponatremia.
- 20 isonatremic regardless the infecting strain, as a control group.

Patients in each group will be selected to match for sex, age, and nutritional status within the following age range :

Test groups patients	Control group patients
12-24 months	+/- 3 months (*)
25-48 months	+/- 6 months
>49 months	+/- 12 months

(*) with the lower limit of 1 year of age

Informed consent will be obtained before admission into the study.

2. Procedure

a) Selected patients will be admitted in the study ward, where usual treatment and rehydration will be provided as required according to the patient's condition. In addition, weight will be recorded, collection of 24h urine will be started and a routine blood sample for electrolytes, glucose, BUN, and creatinine will be drawn if this has not already been done in the general ward.

Stool culture will be performed on a rectal specimen plated at the bed sid.

b) Hydration status will be assessed on clinical criteria: skin turgor, moist of mucosae, haemodynamic condition, weight gained since admission. If the patient is found dehydrated, rehydration will be insured prior to investigations, IV or PO according to the patient's condition. After complete rehydration, the following steps will be taken:

i: Overnight fluid restriction will be initiated after a normal meal, so that the only fluid given (IV or PO) will be to replace stool losses and prevent dehydration.

ii: On next morning, a fresh urine sample will be collected for electrolytes, creatinine and osmolality, and a 5ml sample of blood will be drawn and immediately processed :

- a 2ml aliquot will be utilised for assay of proteins, electrolytes, creatinine and osmolality
- the remaining 3ml will be placed io ice and centrifuged. The plasma will be divided into two parts which will be frozen for eventual assay of ADH (-70°C) and toxin levels (-20°C).

iii: A load of 20ml/kg of plain water will be given over the next 30 minutes and urine output measured every hour for the next six hours. During this time the following tests will be performed :

At H1 : Urine osmolality and creatinine, blood osmolality and creatinine (1.5ml) and blood ADH (1.5ml processed as described above).

At H2 : Urine osmolality

At H3 : Same as H1

At H4 : Same as H2

At H6 : Same as H1

iv: Throughout the study a daily sample of stool will be examined for sodium content. The patient's weight will be recorded daily as well as the exact amount of infused and/or ingested sodium.

In summary, the duration of the study is 24 hours plus a 24 hours allowance for rehydration if the patient is dehydrated on admission. The total volume of blood specifically required is 14ml per patient for the entire study.

In order to avoid multiple venous punctures, a sampling needle can be inserted and left in place after flushing with 2ml of normal saline pushed from a syringe rinced with heparin. This procedure can be used during the water load test.

c) Safety of the procedure :

1. A load of 20ml/kg of plain water induces a moderate fall in plasma osmolality. In normal subjects, plasma osmolality is set at 283+/-11 mOsm/l (4), ranging from 294 mOsm/l in the concentrated phase to 272 mOsm/l in the diluted phase. Assuming that the fluid restriction, before testing, sets the plasma osmolality at the concentrated level, the fall in serum osmolality can be calculated to be about 20 mOsm/l, thus leaving the osmolality within the physiological range. It has also been experimentally demonstrated that the maximum dilution observed with this amount of water does not exceed 2-3% of the base-line state and occurs in 30-60 minutes after water ingestion (4). Elimination of excessive water starts almost immediately. Consequently, the fall in natremia remains below 3mmol/l and does not cause any hazard to the patient.

2. Blood sampling is safe under elementary anti-septic procedure. The volume of blood drawn is negligible in adults, even in case of sever anemia. In the most severe case of malnutrition admissible in the study, in a 1 year old baby, and assuming that the patient's HCT is not below 30%, it represents a drop around 1% in the hematocrit if the whole volume is sampled at a time. Since the sampling will be performed over several hours, the hematocrit changes will be negligible.

3. The dose of heparin used to keep the sampling needle free of clot remains negligible and has no effect on the patient's coagulation.

D. SIGNIFICANCE AND RATIONALE

Hyponatremia is an extremely frequent occurence in Shigellosis, especially with Shigella dysenteriae type 1. However, its mechanisms are still unknown. This poses an almost daily management problem to the physician in cases where the patient is dehydrated and requires considerable amounts of fluid.

It is not infrequent that convulsions occur during the rehydration phase of the treatment, and that peripheral edema develops. Understanding the pathophysiological mechanisms of hyponatremia in these cases could allow one to define more accurate guidelines for management of such patients and avoid severe complications such as seizures.

In addition, the central action of the so-called neurotoxin secreted by Shigella dysenteriae type 1 is unknown. It is unclear whether or not neurotoxin is responsible for the seizures seen in Shigellosis (21).

This study proposes to investigate a possible toxin-mediated mechanism of hyponatremia in these patients, which could lead to an inappropriate secretion of anti-diuretic hormone by the pituitary gland. This would, therefore favour the hypothesis of a brain target for the toxin.

The first step in this protocol will investigate indirect evidence for secretion of inappropriate levels of ADH. A second step will be to ultimately establish a relationship between the serum levels of ADH and circulating toxin.

E. FACILITIES REQUIRED

The present hospital and laboratories are sufficient to carry out this study.

F. ANALYSIS OF DATA

Clinical and biological data will be recorded on a separate and confidential file. All data will be stored on microcomputer diskettes.

Calculations will be performed as follows :

Clearances will be calculated according to the general formula of kidney clearances :

$$\text{Clearance} = \frac{U \times V}{P}$$

Where U = urine concentration of substance
P = plasma " " "
V = urine flow (ml/min)

Glomerular filtration rate and osmotic clearance will be calculated with this formula, and free water clearance will be deducted as shown above.

Sodium balance will be calculated as the difference between input (IV fluids, measured amounts of ORS) and output recorded from urine and stool samples.

Statistics will be performed as required.

G. REFERENCES

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H. SUMMARY

1. This study proposes to investigate the mechanisms of hyponatremia in Shigella infections. The hypothesis of an inappropriate secretion of antidiuretic hormone induced by the shigella toxin will be tested.

2. Risks are negligible and include only those related to blood drawing. Mild discomfort will be caused to the patient by the ingestion of a relatively large amount of water over a limited period of time (30 min).

3. Risks will be minimized by observing usual sterile techniques of blood sampling. The number of pricks will be limited by inserting a sampling needle left in place during the tests.

4. Confidentiality will be maintained by the use of serial numbers instead of names. Data will be recorded on coded micro-computer files.

5. Signed informed consent will be obtained.

6. Does not apply.

7. The potential benefit are, f the patient, a more accurate rehydration phase and, for the society, the understanding of an unknwn phenomenon. This may further lead to therapeutic guidelines to prevent some complications and deaths.

8. This study requires the use of blood, urine and stool samples.

SECTION III - BUDGET

Detailed Budget

1. Personnel services

Name	Position	Period	%	Tk.	US$
Dr. D. PATTE	Pr. Investigator	12 months	25	--	--
Dr.(To be named)	" "	12 months	20		1153

2. Supplies & Material

Per patient	Tk.	Sub. Tot.	
Urine Electrolytes x 3	70	210	
Urine Creatinine x 4	64	256	
Urine Osmolality x 6	75	450	
Blood Electrolytes x 3	70	210	
Blood Creatinine x 4	64	256	
Blood Osmolality x 4	75	300	
Stool Electrolytes x 2	70	140	
x 40 patients=		1822	2803

3. Equipment		0
4. Patients hospitalization : 150 Tk./day x 2 days x 40 patients =	12000	461
5. Outpatient care		0
6. ICDDR,B transport		0
7. Rent		0
8. Printing		50
9. Other contractual services: Provision for ADH and Toxin assays (revisable)		1000
10. Construction & Renovation		0
TOTAL		5467

BUDGET SUMMARY

1. Personnel Services	1153 US$
2. Supplies & Material	2803
3. Equipment	0
4. Patients Hospitalization	461
5. Outpatient Care	0
6. Transport	0
7. Rent	0
8. Printing	50
9. Contractual Services	1000
10. Construction & Renovation	0
TOTAL	5467 US$

Rate : 1 US $ = 26 Tk

CONSENT FORM

You are suffering from dysentery due to Shigella. This disease often provokes disturbances in the composition of blood, which cause hazard especially in young children. We are now studying the mechanisms of one of those, namely the level of sodium, in the course of the disease, and we need your kind cooperation. Understanding the mechanisms of this trouble would allow us to provide more accurate treatment in the future and, hopefully to prevent some major complications.

If you accept to join this study, you will be admitted in our study ward where you will recieve normal treatment until you are fully rehydrated. Drugs will be supplied as your condition requires.

After complete rehydration, you will be asked to drink a fair amount of plain water within half an hour. Then we shall record your urine output and draw a small amount of blood over the next six hours. Dosages will be performed on blood and urine.

Your stay in the hospital will not be increased by this study.

If you do not want to join the study, you will be treated normally as required by your condition.

If you accept, do not hesitate to ask all the questions you feel necessary to your decision and sign this consent form after all your questions have been answered.

Signature or thumb print:_____
(Patient's or parents)

Investigator's signature:_____

Date:_____