REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS IN RESEARCH — Date _____

CRL

87

Principal Investigator M. A. Wahed — Trainee investigator(if any) _____

Application No 78-012 — Supporting Agency(if Non-CRL) _____

Title of study Quantitation of ~~or~~-anti-toxin in order to detect the gastrointestinal Protein Loss in Diarrhoea and Dysentery

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):

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 possibly 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 aborted 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

5. Will signed consent form be required:
a) From subjects (Yes) No
b) From parent or guardian (if subjects are minors (Yes) No
6. Will precautions be taken to protect anonymity of subjects: (Yes) No
7. Check documents being submitted herewith to Committee:
✓ Umbrella proposal - Initially submit an overview(all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (REQUIRED)
✓ Informed consent form for subjects
___ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

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

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

M. A. Wahed
Principal Investigator

Trainee

Return 2 copies of entire protocol to Chairman, Review Board on Use of Human

78-012
Recd 23/3/78

SECTION I - RESEARCH PROTOCOL

1) Title: Quantitation of α_1-antitrypsin to detect the gastrointestinal protein loss is diarrhea and dysentery.

2) Principal Investigator: M.A. Wahed

3) Starting Date: 1.5.78

4) Completion Date: 1.11.78

5) Total Direct Cost: $6,407 with $3,156 as incremental cost.

6) Abstract Summary:

Loss of plasma protein into the G.I. tract may be an important mechanism for certain protein deficiency disorders. Previously available methods to detect the protein loss into the gut are cumbersome, expensive, imprecise and technically hazardous due to use of radio-active-materials and long steady state. A recently developed method uses the presence of α_1-antitrypsin, a protein which resists enzymatic proteolysis in the gut, to determine enteric protein loss. The new method is simple and loss of vascular protein can be measured by comparing the ratio of α_1-antitrypsin in stool and serum. The α_1-antitrypsin can be measured using radial-immunodiffusion (RID) technique. Neither the duration nor the magnitude of protein loss in dysentery (amebic or shigella) is known. After evaluation of the α_1-antitrypsin technique we hope to establish the presence and duration of protein loss in different diarrheal diseases.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

Objective & Background: Loss of plasma protein occurs in colonic inflammatory diseases such as ulcerative colitis and regional enteritis. Gastrointestinal protein loss in shigellosis and amebiasis have never been measured. Similarly, the duration of protein loss in diseases such as shigellosis and amebiasis is unknown. Cholera, as shown at this laboratory, is not associated with protein loss (1). It would seem logical, however, since blood is grossly present in the stool that protein loss also occurs in these diseases. It is also not known whether protein loss occurs in diarrheas caused by V. Parahemolyticus, Rotavirus and toxigenic E.coli.

Previous methods (1,2,3,4,5) of studying gastrointestinal protein loss have required radio-active materials. This introduces considerable technological handicaps and potential health hazards. Also, these methods require several days of steady state conditions to obtain reliable results.

Recently a new method (6) which uses the presence of α_1-antitrypsin which resists enzymatic proteolysis has been developed. The method is the quantification of α_1-antitrypsin as a reliable index to measure plasma protein loss into the gut. α_1-antitrypsin is present in serum in much higher amounts than in intestinal contents. It can be easily measured using radial immuno-diffusion (RID) technique.

Therefore, loss of vascular protein into the intestinal content can be measured by comparing the ratio of α_1-antitrypsin in stool and serum. We have found in a small pilot study that α_1-antitrypsin is not present in cholera (0.8mg/g of stool) but markedly present in shigellosis (14.4 $\pm$ 2.0mg/g of stool) in the acute phase. It has been also observed that in two shigella cases in the stool to blood ratio decreases while the patient was improving. (The normal range of α_1-antitrypsin in plasma is 2.0gm/L to 4.5gm/L).

The new method appears to be simple, safe and semiquantitative.

3. Rationale: α_1-antitrypsin unlike ordinary proteins does not undergo change during the passage through the gut, therefore it may be considered an "ideal" marker for estimating loss of protein from serum.

Loss of protein in stool may have important consequences in states of protein under nutrition. Measurement of this potentially important factor is now feasible with the method described.

B. SPECIFIC AIMS

1. To evaluate the method of the estimation of α_1-antitrypsin in both stool and serum by radial immuno diffusion technique.

2. To determine semiquantitatively protein loss in different diarrheal diseases.

3. To determine the extent and duration of protein loss in those diseases in which protein loss is found.

4. To determine the severity of disease in relation to protein loss, comparing children having clinically severe shigellosis with children having mild illnesses. Similarly, the relationship between proctoscopically determined ulcerative and non-ulcerative amebic colitis and protein loss will be determined.

C. METHODS OF PROCEDURE

Description of Method: Stool will be collected in ice surrounded bucket over a period not exceeding one hour and 1ml blood will also be collected. Samples will be stored in Revco till lyophilised. Estimations on a single patient will be run on the same plate at the same time under standard conditions. The 24 hour volume of wet weight stool will be recorded. Twenty cc's of stool will be lyophilised. The total dry weight after lyophilization will be measured. The lyophilised stool will then be extracted with normal saline at room temperature. The extraction mixture will be intermittently shaken for 30 minutes and then centrifuged at 12000g for 15 minutes at 4°C.

Five μl of stool aliquot and serum (1:10 dilution in N/S) will be loaded in M-partigen plates specified for α_1-antitrypsin determination. Known standards of 40mg%-172mg% will also be run. The plates will be incubated at room temperature for 2 days, after which the precipitin rings formed will be measured. The squares of the diameters of the precipitin rings obtained will be plotted with standards of different dilutions on linear graph paper as a function of the concentrations. Plotting these squared values after the end point of diffusion has been reached will normally result in a straight line, which should intercept the ordinate at 11.0 $\pm$ 2.5mm. The concentration of each protein antigen can then be read directly off the straight line. In the case of serum, the results obtained from the curve should be multiplied by the dilution factor.

The ratio of fecal α_1-antitrypsin (mg/gm dry wt) and serum α_1-antitrypsin (gm/L) will be calculated. The total protein loss will also be calculated.

Description of evaluation process:

Evaluations: The technique will be evaluated using multiple samples from the same normal individuals. Also samples from six normal individuals will be studied to obtain normal variations. Finally stool samples will be divided into separate portions and run at different times to obtain the reproducibility of the method.

Six normal subjects without any history of acute illness will give stool and blood for three consecutive days. The stool specimen in different portions of stool as well as serum collected on the day of stool sampling α_1-antitrypsin will be determined.

After evaluation, 6-8 adults and 6-8 children with shigellosis will be studied for evidence of protein loss during the acute phase of illness. In addition, 6-8 children with clinically severe shigellosis will also be studied. Those found to have protein loss will be studied to establish the duration of such losses after antibiotic therapy. The results will be correlated with the presence of fecal leukocytes, pus cells, guaic, stool N_2, total serum protein, serum albumin and total W.B.C.

Patient Selection

Patients studied will be eight adults and eight children in each of the following condition: shigellosis, amebiasis, E.coli diarrhea, Rotavirus and V. Parahemolyticus (including other NAGS). Two separate stool samples will be collected prior to therapy within a four hour period so that antibiotics will not be withheld for more than four hours. Also studied will be the large group of patients who present with mucus, diarrhea and abdominal pain but in whom no etiologic agent is found.

Proctoscopic examinations will be carried on in all amebic and shigella patients to assess severity. Clinical and laboratory findings will be recorded on separate sheets for analysis.

Those diseases in which protein loss is found will then be studied in a sequential manner. Six to eight patients will be studied in each of the following groups:

Uncomplicated shigellosis: Eight cases of 0-5 yr children and eight adults.

Clinically severe shigellosis: Six cases. Only cases with a history of one week or less will be selected.

Amebiasis: a) Six non-ulcerated amebic colitis adult patients.

b) Six grossly ulcerated amebic colitis adult patients.

NAG: Six adults and six children with acute history of illness.

Amasha (diarrhea with mucus but no blood): Six adults and six children with illness of less than one week's duration.

Collection of stools and blood: Admission, 1st day, 3rd day, and 5th day after admission. If diarrhea continues beyond 7th day we will collect samples every 2nd day until the diarrhea stops. This will usually be the 7th day of hospitalization. If not, patients will be studied as outpatients on day 14. One sample at convalescence stage after formed stool is passed will be collected and this serve as a control against the acute phase. Other laboratory tests: R/S for culture on admission, stool M/E & MIF for parasite contents and TWBC on each day of sample collection. Stool N_2 will also be measured. To determine the albumin level in the serum, electrophoresis will also be done. Samples will be coded and read blind in a lot.

Analysis of results:

Assuming we confirm previous evaluation studies done in New Zealand, we will first determine protein using a ratio of 1.0 as the screening point. Differences of positive or negative protein loss between the diseases will be compared using chi square test. Protein ratio will also be compared between experimental groups using the t test. Regression lines will be determined for the increase in a ratio and duration after therapy. Correlation co-efficients with TWBC, proctoscopic severity, fecal leukocytes and pus cells will be analysed.

D. SIGNIFICANCE

Intestinal protein loss may be important factor in protein undernutrition. Dysentery with its probably associated protein loss is frequently seen in malnourished individuals. The duration of such loss is needed to evaluate its importance. Prognosis and the efficiency of therapy, may be closely related to such losses.

E. FACILITIES REQUIRED

Only eight dozen M-partigen plates will be needed. To do the α_1-antitrypsin estimation, some available space in Biochemistry laboratory will be used. The other tests to be done in clinical pathology and microbiology laboratory will be carried out on routine basis and no special space is necessary.

F. COLLABORATIVE ARRANGEMENT:

None

REFERENCES

1. Lindenbum, J. et. al : Studies of gastrointestinal protein loss in cholera and other acute diarrhoeal illnesses, CRL T.C. Report, 1965.

2. Tongeren, J,Measuring gastrointestinal protein loss, Lancet, July 16, 1966, pp-167.

3. Waldmann, T.A.: Protein-losing enteropathy, Gastro-enterology vol. 50, No. 3, pp-442-443.

4. Parkins, R.A.: Protein-losing enteropathy in the sprue syndrome, Lancet 2:1366-1368, 1960.

5. Stanely, M.M. Plasma protein clearance by the gut: A method of stydying the exudative gastroenteropathies. Am. J. Digestive Diseases, New series, vol. 10, No. 12, 1965, pp-993.

6. Crossly, J. et.al:Simple method for diagnosing protein-losing enteropathies, British Medical Journal, February 12, 1977, pp-428-429.

B. BUDGET SUMMARY

	Category	Taka	Dollars
1.	Personnel	23,577.00	-
2.	Supplies	-	3,156.00
3.	Equipment	-	
4.	Hospitalization	20,000.00	-
5.	Outpatients	-	-
6.	CRL Transport	700.00	
7.	Transportation things	-	300.00
8.	Rent, communications and utilities	-	-
9.	Printing & reproduction	600.00	-
10.	Contractual services	-	-
		44,877.00	3,456.00

Total: $ 6,407.00

Conversion Rate $ 1.00 = Tk. 15.2

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name		% Effort	Annual Salary	Project Requirement Taka	$
Dr M.A. Wahed	Principal Investigator	50%	31,584	7,896	-
Dr Abu Yusuf	Co-Investigator	15%	34,536	2,590	-
Dr M.M. Rahaman	Co-Investigator	10%	116,736	5,837	-
Dr R.H. Gilman	Co-Investigator	5%	-	-	-
One Nurse	-	25%	19,752	2,469	-
One Sr. Lab. Tech.	-	25%	26,784	3,344	-
One Field Assistant	-	25%	11,526	1,441	
			Total	23,577	

2. SUPPLIES AND MATERIALS

Items	Unit Cost	Amount Required
40 cm M-partigen plates (α_1-antitrypsin)	$ 193.00	$ 3088.00
Vials known standards	$ 5.00	$ 30.00
Boxes 5 ml disposable pipett	-	-
Box Electrophoresis membrane	$ 33.00	$ 33.00
Roll Scanning paper	$ 5.00	$ 5.00

3. EQUIPMENT

Nil

		TAKA	DOLLARS
4.	PATIENT HOSPITALIZATION		
	200 patient days*	20,000.00	
5.	OUTPATIENT CARE	nil	
6.	CRL TRANSPORT		
	500 miles land transport	700.00	
7.	TRAVEL & TRANSPORTATION		
		nil	
8.	TRANSPORTATION OF THINGS		
	Import of supplies		300.00
9.	RENT, COMMUNICATIONS & UTILITIES		
10.	PRINTING & REPRODUCTION		
	Mimeographs	300.00	
	Xerox costs	300.00	
11.	OTHER CONTRACTUAL SERVICES		
	nil		
12.	CONSTRUCTION ETC.	nil	

* Some of the patient days will be shared with other protocols e.g. 820321 and 820118.

Quantitation α_1-antitrypsin to detect the gastrointestinal protein loss in diarrhoea and dysentery

CONSENT

The Cholera Research Laboratory Hospital is carrying out studies to determine effective treatment of cholera, diarrhoea and dysentery. Due to gastrointestinal diseases, there is chance of losing protein through G.I. tract. A new method to detect such loses has been established. For this, following tests will be done.

1. To determine amount of protein, 1 ml. blood will be taken on admission, first, third and fifth day after admission. Samples of your stool will be analyzed.

2. Dysentery causes ulceration in the rectum which will be examined proctoscopically.

We would like you to participate in this effort. Please sign if you agree. If you do not, usual treatment will not be hampered. You may withdraw your consent at any time and which will not disturb the routine treatment.

Name of the Patient: _____

Patient No. _____

Signature/thumb impression

Relationship of patient with signatory

Quantification of α_1 antitrypsin to detect the gastrointestinal protein loss in diarrhoea and dysentery.

সম্মতিপত্র

কলেরা গবেষণাগার হাসপাতালে কলেরা, ডাইরিয়া ও রক্তআমাশয় রোগের প্রতিষেধক উদ্ভাবনের জন্য গবেষণা চলছে। পেটের পীড়া হলে ডাইরিয়া বা আমাশয় রোগের সময় প্রোটিন [illegible] শরীর থেকে বের হয়ে যায়। সম্প্রতি [illegible] একটি নূতন পরীক্ষা আবিষ্কার করা হয়েছে, যার [illegible] জানতে পারি কত পরিমাণ বের হচ্ছে এবং তার জন্য নীচের পরীক্ষাগুলি করা হবে।

(১) ভর্তির দিন ও ৩ দিন পর পর (রোগ না সারা পর্যন্ত) সামান্য পরিমাণ (১ সি.সি.) রক্ত নেয়া হবে। ঐ সময় মল ও সংগ্রহ করা হবে। (আলফা-১ পরিমাণ নির্ণয়ের জন্য)

(২) আমাশয় রোগ হলে সাধারণতঃ [illegible] এবং তা ভিতরে নলের দ্বারা পরীক্ষা করা হবে।

এটি নূতন জিনিষ জানার জন্য সাহায্য করবে [illegible] [illegible]। [illegible] [illegible] [illegible]। [illegible] [illegible] ক্ষতি হবে না। [illegible] আপনি যে কোন সময় হাসপাতাল ছেড়ে যেতে পারেন।

টিপ সই —
ও
সহি —

স্বাক্ষর/টিপসই —

স্বাক্ষরকারীর সঙ্গে রোগীর সম্পর্ক

ABSTRACT SUMMARY

Loss of plasma protein into the G.I. tract occurs in several diarrheal disorders. The extent or magnitude of protein loss in shigellosis and amebiasis have never been measured and such losses in E.coli, Rotavirus, V.parahaemolyticus are not known. Previous methods to detect the protein loss into the gut are unreliable, expensive, technically hazardous due to use of radioactive materials and long steady state. Recently a new method which uses the presence of α-antitrypsin which resists enzymatic proteolysis has been developed. Loss of vascular protein can be measured by comparing the ration of α-antitrypsin in stool and serum. The α-antitrypsin can be measured using radial immuno diffusion (RID) technique.

1. a) Six normal subjects for validation of the method.

   b) Eight adults and eight children suffering from Shigellosis, E.H., E.coli diarrhea, Rotavirus, V.Parahaemolyticus and "Amasha".

   c) Those disease in which protein-loss is found will then be studied in sequential manner. 6-8 patients will be studied in each of the following groups.

      Uncomplicated Shigellosis: One group is children of age 0-5 yr and the other group is adults.

      Clinically severe Shigellosis: Both adult and children.

      NAG: Both adult and children

      Amebiasis: 6 non-ulcerated amebic colitis and 6 grossly ulcerated amebic colitis.

2. No potential risks.

3. Not applicable.

4. Not applicable.

5. Though, there is no such potential risks still we will obtain a signed consent from the subject or from the guardians in case of minor because we will collect small qualtities of blood.

   a) Not applicable.

   b) Not applicable.

6. No involvement of interview except to obtain the history of illness which is done routinely.

7. Intestinal protein loss may be important factor in protein undernutrition. Dysentery with its probable associated protein-loss is frequently seen in malnourished individuals. The duration of such loss is needed to evaluate its importance. Prognosis and the efficacy of therapy may be closely related to such loss. So, there will be general benefit to these patients.

8. We shall use patient records. We shall only take 1 ml blood and stool at several intervals. Proctoscopy will be done to see the ulceration in the rectum, if any.