REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

60

Principal Investigator Mrs. Khaleda Haider Trainee Investigator (if any)

Publication No. 79-011 Supporting Agency (if Non-ICDDR,B) I.N.F.S., D.U.

Title of Study Microbiological, Biochemical Anthropometric correlates of children with ~~ed nutrition during bacillary 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 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 NA
(d) Sensitive questions Yes No NA
(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 Board:
___ 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.

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

Khaleda Haider
Principal Investigator

Trainee

79-011
Rec'd 12/9/79

SECTION I - RESEARCH PROTOCOL

1. Title : Microbiological , Biochemical and Anthropometric correlates of children with altered nutrition during bacillary dysentery.

2. Principle Investigator : Mrs. Khaleda Haider, ICDDR,B Fellow on Microbiology Branch

3. Starting Date : September 1, 1979.

4. Completion Date : December 31, 1980.

5. Total Direct Cost : Tk. 73,000

6. Abstract Summary :

The purpose of this study is to enumerate the bacterial flora of normal individual and the changes in these flora during malnutrition with bacillary dysentery. Children of the age group below six years of age are mostly affected by malnutrition in our country. Total hundred fifty samples will be included in this study. Of them fifty will be severely malnourished with basillary dysentery of the age group 3 - 6 years of any sex and fifty normal healthy children with dysentery of the same age group and another fifty normal healthy children of comparable age group. Children of all the groups will be chosen from the same area. All the patients will be chosen from the Treatment Centre of ICDDR,B. Patient will be selected by doing stool microscopic examination. Samples will be collected before giving any antibiotic treatment.

There are lots of biochemical deficiencies in this age groups of children which may alter the bacterial flora. 4-5 cc blood will be drawn for measuring Vitamin-A, Total protein, Albumin, Globulin, Hb and Heamatocrit values. Patient will be interviewed and their clinical symptoms will be noted and anthropometric measurements will be taken for finding out the nutritional status. Throat swab will be taken to find out the throat flora. Jejunal aspirate will be drawn for bacteriological study.

The guardian will be informed about consent form and if agreed will be asked to sign it before the child is taken in the study. They will be informed about the follow up visit after four weeks when they will be picked up from the house.

The study does not involve any risk to the subject and during the period of study (first visit and follow up), the house will be visited for any illness and will be asked to report if there are any. The scientific benefit will be manifold and will give enormous data in the field of study.

7. Review :

a. Research Involving Human Subjects : _____

b. Research Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective : The main objective of this research protocol is to find out

   a. whether there is a different kind of aerobic bacterial flora in well nourished and malnourished dysentery patients and how it compares with normal healthy individuals.

   b. whether there is a different distribution of enteric aerobes between well-nourished and malnourished dysentery cases.

   c. what are the specific differences in Vitamin A, Total protein A/G ratio and Haemoglobin and other biochemical parameters in serum between the groups.

   d. whether there is a difference in carrying resistance to Ampicillin and other antibiotics between well and malnourished children.

2. Background :

Malnutrition and diarrhoea the two major health problem of Bangladesh are so closely related that it is impossible to seperate them completely(1,2,3). There is undoubtedly an effect of diarrhoea on the different biochemical changes in malnutrition and vice versa. Poor social and economic conditions predispose to both diseases, and each disease aggravates the other (4). The

result of the two disease combined in the same child is complex, for example loss of potassium and magnesium due to diarrhoea increases the intracellular electrolyte depletion of a malnourished child, whereas the loss of water in fluid stools may mask the overhydration which is characteristic feature of malnutrition.

It is a general understanding that malnourished children are more susceptible to different disease as they have less antibodies with them. Protein energy malnourished children with diarrhoea had a tendency to show more bacteria in the stomach and jejunum than those without diarrhoea(5). Again the gram negative anaerobic bacilli(bacteroides) to be more common in the jejunum in the absence of diarrhoea(5). Anaerobes found predominating over aerobes in the control subjects and a striking change in the proportion found in the children with diarrhoea. Enterobacteria were found in almost all diarrhoeal samples and in the group of children as a whole they predominated over any other type of organisms(6). It can now be summerized that healthy babies have more anaerobes and the diarrheoa patients have more aerobes.

Differences in fecal flora were not found in relation to respiratory infection, uncomplicated measles and whooping cough, or in exanthematic diseases or skin infections(6). It has already been found that patient with shigellosis, the anaerobic components

decreased to levels comparable to those on the facultative flora. Some informations are yet to know about the same patients but wellnourished and malnourished.

3. Rationale :

The rationale behind doing this research is to find out the label of bacterial flora in normal and malnourished children of Bangladesh. Better understanding of the bacterial flora and its association with Bacillary dysentery and malnutrition will result in understanding the specific reasons of the disease (causative organism) resulting in better treatment.

B. SPECIFIC AIMS

The specific aims of this research are :

1. To compare the distribution of aerobic flora in throat, Gut and jejunal aspirate of malnourished dysentery patients, welnourished dysentery patient and welnourished healthy children of the comparable age groups.

2. To find out the nutritional status of the malnourished and well-nourished children.

3. To find out the sensitivity pattern of Shigella, E. Coli and other pathogens to Ampicillin and other antibiotics and to screen the R. factors present in those organisms.

C. MATERIALS AND METHODS :

Three groups of children of age group 3-6 years will be included in this study.

First group : Well-nourished children reporting to the hospital with bacillary dysentery.

Second group : Severely malnourished children reporting to the hospital with bacillary dysentery.

Third group : Normal well-nourished children of comparable age group and social status.

Microbiological technique :

Fecal materials obtained by sterile rectal catheters will be collected directly into a sterile bottles before giving any antibiotic to the patient. Microscopic examination of stool will be done for the selection of bacillary dysentery cases. Patient with an acute history of fever and passing mucoid or bloody stools will be selected for the study, provided their guardian understand and sign the consent form. The specimens will be brought quickly to the laboratory. Dilution and inoculation of bacteriological plates will be completed within 30 minutes of collection.

Fecal samples will be 10-fold serially diluted in sterile saline from concentrations of 10-1/ml to 10-6/ml. 0.1 ml aliquot of these dilutions will be cultured by surface plating onto gelatin agar, MacConkey, Blood Agar, SS, Monsur's SP and TCBS plates in appropriate dilutions. All the media will be inoculated at $37^{o}C$ for 18 hours.

Representative lactose fermenting and non-lactose fermenting colonies will be picked up and confirmed by different biochemical and serological analysis. Results will be expressed as the number of organism per gm.of feces. At least two colonies of each of shigella species, E. Coli and other pathogens will be stored on blood agar base slant for finding out the sensitivity pattern to Ampicillin and for Toxin assay.

Throat swab will be taken and plated on Blood agar, MacConkey and chocholate agar media and incubated aerobically and in presence of carbondioxide.

Antibiotic Sensitivity Pattern :

Sensitivity pattern of the pathogenic organisms isolated from all the patients and normal individuals will be tested by routine Kirby Bauer method(7). Standard growth of the organisms will be inoculated onto Muller Hinton medium and the BBL antibiotic discs containing specified concentration of antibiotic will be placed on the plates at certain distances. The zone size of inhibition if any, will be measured and compared with the control.

Biochemical determinations :

Blood will collected in heparinized tube for haemoglobin. Non-heparinized tubes will be used to collect blood for albumin, globulin, Total protein and Vitamin A.

After collection blood will be centrifuged at 3000 x g for 20 minutes to collect the serum and to seperate plasma from the red colls. Total protein will be determined by Goldenburg Refractometer Albumin and globulin will be determined by dye binding method(8). Vitamin A will be determined fluorometrically(9). Serum electrolytes will be done using standard methods followed in our centre.

National status :

Anthropometric is one of the most important indices to find out the nutritional status of an individual. We shall use Harvard values(10) as standard for comparison.

Anthropometric parameters which will be included in this study are height(cm), weight(kg), sex, age, skinfold thickness(mm), head circumference(cm), chest circumference and arm circumference. During the course of the studies on children admitted to hospital, physical examination of the patient will be conducted with a view to detect signs and symptoms of malnutrition. All the patients will be used for a follow up study after 4-6 weeks. They will be brought to the hospital and stool, blood, throat swab and jejunal aspirate will be collected for microbiological and biochemical studies as done earlier and nutritional status will be determined.

D. Though the problem of malnutrition has been studied in detail its relationship to bacterial population in the gut, throat & jejunal aspirate during diarrhoeal illness at different stages of the disease has not been fully documented. This study will surely throw light on the effect of altered bacterial population during malnutrition on bacilary dysentery. The different biochemical labels in the blood during dysentery of the malnourished children will show a relationship between balanced or unbalanced food with diarrhoeal illness.

E. FACILITIES REQUIRED:

1&2 Office space willn not be required for this study. Laboratory space is already in existance. A portion of the microbiology laboratory table and some working time in the Biochemistry Branch is required. No new extra space will be needed.

3. No hospital space will be required. All samples will be collected from the patient in outdoor. No admission will be required. Blood samples will be collected by staff nurses or any Dorctor on duty.

4. For toxin assay the following animals will be needed. Infant mouse 400 newborn swiss albino suckling mice(2-4 days old).

5. For follow up study all the patients (50) will be grought to the hospital.

6. All the major equipments are already is use in the Chemistry and Microbiology laboratory, ICDDR,B. Some of the equipments will be brought from Institute of Nutrition and Food Science, Dacca University. Part of the studies will be done at the laboratory of the Institute of Nutrition, Dacca University.

7. Specialised equipments : None

8. For follow up patients some liquid oral vitamins will be needed 100 Ph.

COLLABORATIVE ARRANGEMENTS:

Some equipments will be brought from the institute of Nutrition, Dacca University for some Anthropometric measurements. Biochemical analysis will be done both in Biochemistry Laboratory of ICDDR,B and in the Nutrition Laboratory, Dacca University. For dietary surveyyone experienced personal (Dietecian) will be brought from Institute of Nutrition, Dacca University.

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9. Hansen, Leland G., and Warwick, Warren J. : An improved assay method for serum Vitamin A and E. using fluorometry Am. J. Clin. Pathol. 70,922-923,1978.

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INFORMATION TO PATIENT AND CONSENT FORM

The diarrhoea you have, may be caused by various pathogenic organisms namely V. cholerae, Shigella, Salmonella, E. Coli. We are studying these bacteria to learn what kind of disease it causes and how they make people sick. We also want to find out whether malnutrition helps these type of infection.

We hope this study will help in treating and preventing the disease in the future.

We are asking you to join our study because, you or your child is possibly infected by any of the above said bacteria. If you agree to join, we will take some simple measurements like height, weight, skinfold thickness etc. and also take a small amount of blood from your arm for laboratory tests. You will be treated and discharged in the same way as other patients. After four to six weeks you will have to return to the hospital for a short time so that we can take another sample of your blood and stool for re-examination and can take some measurements of height, weight etc. If you need any other treatment or any medicine you will be given properly, we shall send a car to pick you up and drop you back. All the information will be kept strictly confidential.

CONSENT : I have read or heard the information given above and I am willing to help you by joining this study or by allowing my child to join.

Signature of Patient ..... Date .....

Signature of Parent or Guardian ..... Date .....

Signature of Principal Investigator ..... Date .....

রোগীর জন্য তথ্যাবলি এবং সম্মতি পত্র

আমাশয় আমাদের শিশুদের একটি প্রধান রোগ। আমাশয় এর সকল জীবাণু মানুষকে কিভাবে আক্রমন করে এবং অসুস্থ করিয়া ফেলে তাহা জানিবার জন্য আমরা একটি পরীক্ষা চালাইতেছি। আমরা আরও দেখিতে চাই যে, অপুষ্টি এই জীবাণু দ্বারা আক্রমন হইতে সাহায্য করে কি না। আমরা আশা করি এই পরীক্ষা চালাইয়া আমরা ভবিষ্যতে এই সকল জীবাণুর আক্রমণ হইতে শিশুদের কি ভাবে রক্ষা করা যায় তাহা খুঁজিয়া পাইব।

আমরা আপনার শিশুকে এই পরীক্ষায় যোগদানের জন্য আবেদন জানাইতেছি। কারণ আপনার শিশু উপরোক্ত জীবাণু দ্বারা আক্রমন হইয়াছে। আপনি যদি যোগদানে ইচ্ছুক থাকেন তাহা হইলে আমরা আপনার শিশুর কতগুলি খুবই সাধারণ মাপ নিব যেমন- উচ্চতা, ওজন ইত্যাদি এবং পরীক্ষার জন্য সামান্য রক্ত নিব। আপনার শিশুকে অন্যান্য রোগীদের মত চিকিৎসা করিয়া ছাড়িয়া দেওয়া হইবে।

চার সপ্তাহ পর আপনার শিশুকে আবার হাসপাতালে আসিতে হইবে এবং আমরা পুণরায় আপনার শিশুর রক্ত ও পায়খানা পরীক্ষা করিয়া দিব। এই জন্য আপনাকে এবং শিশুকে আমরা গাড়ী দিয়া আনিব এবং আবার গাড়ী দিয়া বাড়ী পৌছাইয়া দিব। এই সকল পরীক্ষা আমরা বিনা পয়সায় করিব। যদি আপনার শিশুর কোন ঔষধ লাগে তবে তাহা দিব এবং অন্য কোন চিকিৎসা লাগিলে তাহা করিব। আপনার শিশুর সকল তথ্য গোপন রাখা হইবে। আপনি ইচ্ছা করিলে এই পরীক্ষা হইতে যে কোন সময় আপনার শিশুর নাম কাটাইয়া লইতে পারেন।

সম্মতি

আমি উপরোক্ত সমস্ত তথ্য পড়িয়াছি বা শুনিয়াছি এবং আমি আপনাকে সাহায্য করিবার জন্য স্বেচ্ছায় আপনার পরীক্ষায় আমার সন্তানকে যোগদানের সম্মতি দিলাম।

রোগীর স্বাক্ষর----------

পিতা মাতা বা
অভিভাবকের স্বাক্ষর----------

প্রধান গবেষণাকারীর
স্বাক্ষর----------

তারিখ----------

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	Percent of effort or number of days	Annual Salary	Project Requirements TAKA	Project Requirements DOLLARS
Mrs. Khaleda	Research fellow	100	Tk. 18,000		
Un-named	Dietician	10	(to be brought) (from INFS,D.U.)		

2. SUPPLIES AND MATERIALS

Items		Total Cost
Media	Tk.	2400.00
Chemicals	Tk.	1500.00
Lab. Supplies	Tk.	1000.00
Glass & Plastic ware	Tk.	1000.00
Biochemical & other tests	Tk.	6000.00
Animals	Tk.	1200.00
Office supplies	Tk.	300.00
Medicine	Tk.	1000.00
Pathological tests	Tk.	800.00
Total	Tk.	14,000.00

3. EQUIPMENT

None

4. PATIENT HOSPITALIZATION

None

5. OUTPATIENT CARE

Number of Outpatients

50 X 2 = 100 Tk. 5000.00

6. CRL TRANSPORT

Mileage - Dacca: 600 miles Tk. 2,000.00

7. TRAVEL AND TRANSPORTATION OF PERSONS

None

8. TRANSPORTATION OF THINGS

None

9. RENT, COMMUNICATIONS & UTILITIES

None

10. PRINTING AND REPRODUCTION

Tk. 1000.00

11. OTHER CONTRACTUAL SERVICES

None

12. CONSTRUCITON, RENOVATION, ALTERATIONS

None

B. BUDGET SUMMARY

	Category	Year 1		Year 2		Year 3	
		Taka	Dollars	Taka	Dollars	Taka	Dollars
1.	Personnel	18,000	-	18,000	-		
2.	Supplies	14,000	-	10,000	-		
3.	Equipment	-	-	-	-		
4.	Hospitalisation	-	-	-	-		
5.	Outpatients	5,000	-	4,000	-		
6.	CRL Transport	2,000	-	1,000	-		
7.	Travel Persons	-	-	-	-		
8.	Transportation Things	-	-	-	-		
9.	Rent/Communication	-	-	-	-		
10.	Printing/Reproduction	-	-	1,000	-		
11.	Contractual Service	-	-	-	-		
12.	Construction	-	-	-	-		
	Total:	39,000	-	34,000	-		

INFORMATIONS ON ABSTRACT SUMMARY

1. It has been observed from the records that children under 6 years of age are mostly affected by malnutrition which results in many cases dysentery syndromes. As our programme is to find out the relationship between malnutrition and dysentery and also to find out the antibiotic resistance in dysentery due to malnutrition we opted to study children under six years of age.

2. The study includes only interviewing the patients and their parents and taking approximately 5 cc. venous blood. There is no potential risk involved in this study.

3. Not applicable.

4. The data will be collected and will be analysed by statistical methods by the investigator herself.

5. a. Though it does not involve potential risk a consent form in Bengali will be shown to and signed by the parents/wards of the patient.

   b. No information will be kept withheld from the patients.

   c. Not applicable.

6. The patient or his parents/wards will be interviewed for Dietary information and social status by the investigator with the help of a qualified dietician who will be brought from the Institute of Nutrition, Dacca University. Whole interview will take about an hour.

7. The potential benefit obtained from this study will be to find out the data on the relationship between malnutrition and dysentery and also label of antibiotic resistance during dysentery during malnutrition; for which we do not have quite convincing research data.

8. Not required.