22

Date 28.05.85

ETHICAL REVIEW COMMITTEE, ICDDR,B.

pal Investigator MS. ASMA HOSSAIN

ation No. 85-0015(P)

f Study "MICROBIOLOGICAL PROFILE NFANTILE DIARRHOEA"

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

Project status: PILOT STUDY

( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

urce of Population:

Ill subjects (Yes) No
Non-ill subjects Yes (No)
Minors or persons under guardianship Yes (No)

s the study involve:

Physical risks to the subjects Yes (No)
Social Risks Yes (No)
Psychological risks to subjects Yes (No)
Discomfort to subjects Yes (No)
Invasion of privacy Yes (No)
Disclosure of information damaging to subject or others Yes (No)

s 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)

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)

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.

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

l Investigator

85-015(P)
28/5/85aa.

SECTION I - RESEARCH PROTOCOL

1. Title : "Microbiological profile of infantile diarrhoea"
2. Principal Investigator : Asma Mossain
3. Starting Date : MID JUNE,1985
4. Completion Date : MID DECEMBER,1985
5. Total Direct Cost : US$1160.00
6. Availability of Funds :

a. Scientific Director's Remarks :

The protocol has been approved by DTWG.

Sd/- Dr.D.A. Sack
19.5.85

7. Abstract Summary :

This study will be carried out with diarrhoeal infants with age ranging from 0-1 year, of low socio-economic status, with illness of 1 to 5 days' duration, sufficient to require admission to a hospital. Their fresh stool samples will be assessed for the prevalence of enteric pathogenic bacteria, parasites and rotavirus. The incidence of isolated faecal strains of the diarrhoeal (well-nourished and malnourished) infants will be compared age for age with non-diarrhoeal infants serving as control.

Programme Head, Disease Transmission Programme:

David A. Sack DTP

(Dr. David A. Sack)

8. Reviews :

a. Research Involving Human Subjects : _____

b. Research Review Committee : _____

c. Director : _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective

The main objective of this protocol is to find out :

a. The nutritional status of the infants anthropometrically

b. The prevalence of pathogenic organisms in the diarrhoeal (well-nourished and malnourished) infants

c. The prevalence of the enteric bacterial flora in the diarrhoeal (well-nourished and malnourished) infants and non-diarrhoeal infants.

d. The antibiogram of the pathogenic organisms.

2. Background

The problem of fatal, infantile diarrhoeal disease is being considered as a major cause of death in infancy and early childhood among the economically underprivileged populations of the world(1).

According to Hardy and Schlissmann, the acute diarrhoeal diseases are estimated to account for about 5 million deaths of infants and children each year throughout the world(2). In children under 5 years of age, diarrhoeal disease was the principal or major cause of death in most of the reporting Latin American countries in 1954, with rates upto 150 times greater than that obtaining in the U.S.A.(3). In Egypt, districts with health bureaus reported that the infant mortality in 1949 was 134 per 1,000 live births. Of 64,914 infant death in which a cause was reported 54% (35,083) were attributed to "diarrhoea and enteritis" (4). Maslov and Grechishnikova, who reported that in 1913 in

Russia infant mortality was 273 per 1,000 live births with approximately one-third attributable to gastro-intestinal deaths while in 1956 in Leningrad infant mortality was 45% per 1,000 with only 4% of the total attributable to gastro-intestinal diseases(5). In a review Hardy concluded that diarrhoeal disease - one of the world's oldest problem- remains today the chief killer of infants in many lands, the mortality affecting predominantly children under 2 years of age.(6).
In Indonesia, each child is likely to experience one or two attacks of diarrhoea per year during the first three years of life (Rohde & Northrup, 1976). (7).

Diarrhoea still remains as one of the major cause of mortality and morbidity in infants and children in the developing countries like Bangladesh. About 35% of all deaths in children are attributed to diarrhoea. Moreover as 75% of children under 3 years suffer from protein energy malnutrition (PEM) and diarrhoea-PEM-Infection makes a vicious cycle, the incidence and severity of diarrhoea continues to remain high. In Bangladesh the average attack rate is 3 attacks per child annually (8).

Acute diarrhoea is an important determinant of malnutrition and the main cause of death among young children (9).

In less developed countries, where diarrhoeal disease is highly prevalent a recognized synergism with nutrition seriously affects general health (Scrimshaw, 1959). The resultant deterioration in nutritional status, frequently to the level of malnutrition, is

reflected in impaired resistance to other infections and the precipitation of specific nutritional disorders, notably Kwashiorkar(10).

Diarrhoea is more prevalent in infants who are artificcially fed, in those of lower socio-economic status and in those who are malnourished. The morbidity and mortality of gastrointestinal disease is also greater in malnourished than in well-nourished infants (Jadhav, 1965; James, 1972) (11).

The early invasion of intestinal tracts of new born infants by bacteria, either ascending or descending, often within ten hours after birth and before feeding, first observed microscopically by Breslau in 1866, was confirmed by Billroth in 1874 and Nothnagel in 1881 (12).

The human enteric tract is subject to infection or infestation by many pathogenic bacteria, viruses, protozoa and helminths, but the impression gained from a previous review is that the Shigella bacteria are presumably the chief pathogenic agents of morbidity and mortality from diarrhoeal disease in populations living under conditions of poor sanitation and hygiene. Schliessmann[6] worked on that "in areas of high endemicity there is substantial evidence that the primary cause of acute, infections diarrhoeal disease is infection with a species of the genus Shigella (14). Ordway concluded that in young infants, particularly in the first 6 months of life, the infecting agents most likely to be causative, are E. coli types and viruses (13). Sabin concluded that particularly in children under 2 years of age, the age group that is

most important from the point of view of mortality, the Shigellae and other specific bacterial pathogens, while still important, may frequently constitute only a small proportion of the aetiologic agents.

As regards Salmonellae, Hardy concluded that while these organisms may cause serious diarrhoeal disease in children, the relative frequency of such infections was unknown. The aetiologic role of certain serotypes of pathogenic E. coli, particularly in nursery groups of newborn children in well-sanitated countries, was acknowledged by Hardy(6).

The studies in Guatemala showed that Entamoeba histolytica, other intestinal parasites as well as pathogenic E. coli were recovered just as often from properly matched control as from children with diarrhoea, while Shigella organisms, although found in only 13.5% of their 201 patients, were recovered less frequently (6%) from the 215 matched controls(15). A carefully controlled study by Ramos-Alvarey et al. showed that enteric viruses as the sole pathogens recoverable by human kidney cultures, were found 3.5 times more often in infants and young children with diarrhoea than in those without; moreover, while enteric viruses were found in 57.5% of the patients, Shigellae and Salmonellae were isolated from a total of only 24.5%(1). In a virologic bacteriologic and parasitologic study on 29 infants with gastroenteritis in Puerto Rico, Young et al. isolated viruses from 14, E. coli serotypes 0111:B4 and 055:B5 from 8, and Shigella, Salmonella and E. histolytica - each from one patient but in each instances associated either with a virus or with E. coli, 0111:B4(16).

Faeces from children (aged from one month to 12 years) with acute diarrhoea admitted to hospital in yogyakarta, Indonesia, from June 1978 to June 1979, were examined for the presence of enteric pathogens. One or more recognized enteropathogens were identified in 56% of children. Rotaviruses were identified in 38% of all children. Toxigenic coliform (predominantly E. coli) were isolated from 21% of children. Salmonella sp. (6%), Shigella sp. (4%) and enteropathogenic parasites (predominantly Trichuris trichurial from 3.5% of children. Mixed infections with two or more enteric pathogens were found in 7.6% of children. Enteropathogenic parasites appeared in increasing frequency with age. They were more common in artificially fed children and in children from families of low socio-economic level. The occurrence of multiple infection with mixtures of enteric pathogens increased with increasing age. Mixtures of parasites and other enteric pathogens only occurred in children with acute diarrhoea(7).

Clinical features and therapy are to be a great extent related to the types of organism and their mechanisms of causing diarrhoea.With the modern techniques in 80-85% causes of diarrhoea aetiological agents can be isolated. The incidence of diarrhoea is reported in different countries shows much variation. Black et al reported E. coli as most common cause of diarrhoea in children aged less than 2 years, Shigella was the second and rotavirus was the third enteropathogens. Among the other pathogens Salmonella and Campylobacter are important.

According to report based on aetiological break-up of diarrhoeal incidences at Matlab rural hospital in 1978-79 for 0-2 years infants were as follows -

Rotavirus 45%, E. coli 28%, NAG vibrios 8% and others 8%(19).

Enteric pathogens associated with diarrhoea were studied for two years at a diarrhoea treatment centre in rural Bangladesh. ETEC was the most frequently identified pathogen for patients of all ages. Rotavirus and ETEC were isolated from ~50% and ~25%, respectively of patients less than two years of age. A bacterial or viral pathogen was identified for 70% of these young children and for 56% of all patients with diarrhoea(20).

Diarrhoeal diseases along with malnutrition is the principal cause of death of the infants and children in Bangladesh. The effect of nutritional status on the frequency and severity of infections disease in a child and the influence of these infections on the child's' growth constitute considerations of great importance in human development.

Scrimshaw reviewed the evidence of nutrition infection interactions and showed that for bacteria, rickettia, intestinal protozoa and helminths, the interaction is mutually aggravating and particularly so in cases of bacterial infection. Malnutrition due to protein deficiency is frequently seen to invite infection(21).

Recurrent infections in early childhood may lead to the development of malnutrition. The simultaneous presence of malnutrition and infection, specially diarrhoea, produce a result more damaging to the host than the combined effect of the two acting independently. In other words infection can aggravate malnutrition and malnutrition can lower resistance to

infection. When either of these entities becomes established and the other is superimposed upon it, a vicious circle of mutual aggravation can be established which may eventually prove fatal. The incidence of the common enteric pathogens in diarrhoeal (well-nourished and malnourished) infants under 1 year, as well as the frequency of the facultative anaerob bacterial flora of both diarrhoeal (well-nourished and malnourished) infants, age for age has not yet been worked out in Bangladesh.

3. Rationale :

The rationale underlying this research protocol is to isolate the various aetiological agents - bacteria, rotavirus and parasites from the stool samples of diarrhoeal (well-nourished and malnourished) and non-diarrhoeal control infants. Also to look for the sensitivity of the pathogens against common antibiotics.

SPECIFIC AIMS

The specific aim of this protocol is to :

1. Compare the nutritional status of well-nourished and malnourished infants with diarrhoea anthropometrically.

2. The incidence of pathogens causing diarrhoea in well-nourished and malnourished diarrhoeal infants.

3. Determine the difference in the facultative anaerobic bacterial flora of the faeces of diarrhoeal (well-nourished and malnourished) infants and non-diarrhoeal infants, age for age.

4. Determine the sensitivity of the pathogens against common antibiotics.

C. METHODS OF PROCEDURE

Three groups of infants ranging from 0-1 year from low socio-economic status will be selected for the study. For any particular age, a sample will be taken for each of the three groups. The infants of group I and group II will be identified from the General Ward of ICDDR,B hospital. Infants of group III will be obtained from a locality at Bashabo.

Group I: Well-nourished infants with diarrhoea

Group II: Malnourished infants with diarrhoea

Group III: Non-diarrhoeal infants

None of the infants should have any antibiotics before taking the sample. Each group will comprise of 25 infants.

1. Selection of subjects :

All the infants will be selected on the basis of the following anthropometric measurements using Harvard standard as reference(22).

Weight : Body weight will be measured using a salter scale. Shoes and outer clothings will be removed before weighing.

Height : Supine length of infants will be measured at described by Jellife(23). Height will be measured to the nearest 0.1 cm using a wooden measuring board.

2. Stool specimens

Stool will be collected in a sterile container in order to find out the facultative anaerobic bacteria, rotavirus and parasitic agents.

3. Bacteriological analysis

Enteric bacteria of both groups of infants will be determined by spreading a swabful and streaking of the faeces on (24)

T.T.G.A. (Monsur's medium) sp.

MacConkey Agar(MA)

Salmonella Shigella Agar(SS)

Brucella Agar (BA)

These plates except BA plate will be incubated at 37°C for 18-24 hours for isolation of the various enteric organisms. BA plates incubated at 42°C for 48 hours in a candle jar. Isolated colonies of different morphology obtained from a single sample will be identified through conventional biochemical reactions (24,25).

4. Virological analysis

About 1 gm of stool sample willbe transferred to phosphate Buffer saline and frozen at -20°C - -70°C for the subsequent identification of rotavirus by ELISA technique (26).

5. Parasitological analysis

A fraction of the fresh stool sample will be examined under a microscope for the detection of parasites.

6. Enteropathogenicity of E. coli (EPEC)

The slide agglutination with commercial available antisera will be used to detect the enteropathogenic serotypes of E. coli (24).

7. Enterotoxicity of E. coli (ETEC)

Test of enterotoxicity of E. coli strains will be conducted in various models as follows :

i. Heat-labile enterotoxin : This will be detected by chinese hamster ovarian cell line (CHO) method (27).

ii. Heat-stable enterotoxin. This will be "done in suckling mice assay" (28).

8. Detection of antibiotic sensitivity.

Disc diffusion method : All the pathogenic strains isolated from the infants will be tested for sensitivity against common antibiotics following the single antibiotic disc method (29). The following antibiotics will be used : Ampicillin, Streptomycin, Tetracycline, Gentamycin, Kanamycin, Septrin and Chloramphenicol.

9. Analysis of Data

Data will be analyzed considering the following points.

i. Stool samples of infants of various ages under 1 year will be collected.

ii. Parallel study will be done by taking the isolated strains from each of 25 infants of Group I, Group II and Group III, age for age.

ii. From the isolated pathogenic strains the antibiotic sensitivity will be determined.

All of which will show us the incidence of the various pathogens present in the diarrhoeal infants and the frequency of the facultative anaerobic bacteria of a particular age under 1 year in the three groups of infants.

D. SIGNIFICANCE

This study will enable us to find the incidence of various enteric pathogens responsible for causing diarrhoea in well-nourished and malnourished diarrhoeal infants of Bangladesh under 1 year. This study will help us to visualize any difference in the facultative anaerobic enteric bacterial flora isolated from infants of the three groups for any particular age below 1 year.

E. FACILITIES REQUIRED

1. Office space : N.A.

2. Laboratory space : ICDDR,B Training Laboratory will be required for most of the study period.

3. Hospital Resources : 0-1 year infants of ICDDR,B General Ward will be required.

4. Animal Resources :

5. Logistical Suppoet : N.A.

6. Major items of equipment : N.A.

7. Other specialized requirements : N.A.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% time used	Annual salary	Project Requirement Taka	US Dollar
Ms. Asma Hossain	Principal Investigator	100%			
Dr. Imdadul Huq	Consultant				
Dr. K.M.S.Aziz	Consultant				

2. SUPPLIES

	Amount required	Unit cost	
Culture Media			Tk. 17,631.25
Antibiotics			Tk. 1,000.00
ELISA test			Tk. 1,350.00
EPEC test			Tk. 9,000.00
		Sub total	Tk. 28,981.25

3. EQUIPMENT - Nil

4. PATIENT HOSPITALIZATION - Nil

5. OUTPATIENT CARE - Nil

6. TRANSPORTATION OF PERSONS - Nil

7. TRAVEL AND TRANSPORTATION OF PERSONS - Nil

8. TRANSPORTATION OF THINGS - Nil

9. RENT, COMMUNICATION, UTILITIES - Nil

10. PRINTING AND PUBLICATION - Nil

11. OTHER CONTRACTUAL SERVICE - Nil

12. CONSTRUCTION, RENOVATION, ALTERATION - Nil

B. BUDGET SUMMARY

Category	Taka	US Dollar
1. Personal		
2. Supplies	Tk.28,981.25	
3-12 None		
Total	**Tk. 28,981.25**	-

= $ 1159.25

Conversion rate $ 1.00 = Tk.25.00

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