"CULTURE NEGATIVE" DIARRHOEA: EPIDEMIOLOGICAL AND CLINICAL OBSERVATIONS

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Introduction

Little is known about the incidence or clinical picture of diarrhoea caused by toxigenic strains of E.coli, mainly due to lack of laboratory techniques to identify these toxigenic strains. Recently devised techniques are available for identifying toxigenic strains of E.coli, which enables us more extensive epidemiological and clinical evaluation of Toxigenic E.coli.

Background and Objective

Some of the cases coming to our Matlab hospital for which no known bacterial pathogen can be determined, may be due to toxigenic E.coli. With the existence of an excellent system of follow-up of diarrhoeal cases, a prospective study of these "culture negative" cases was undertaken to define epidemiological and clinical parameters of toxigenic E.coli diarrhoea.

This report of the study is preliminary describing only progress upto date, since positive identification of toxigenic E.coli strains remains pending for technical reasons.

Materials and Methods

Initially, all diarrhoea cases received at Matlab Hospital with rectal swab cultures negative for cholera, shigella and salmonella, but non-participants in 1974 cholera toxoid field trial were selected as index-cases. Later, with the suggestion that cholera toxoid would not substantially interfere with E.coli diarrhoea, index cases were taken from participants of toxoid trial also, who otherwise qualified as culture negative diarrhoea case. Controls will be identified from among these cases, whose stool does not contain any toxigenic E.coli.

A total of fifty cases were studied. On admission,

patients were put in routine hospital procedures for recording admission clinical status along with a careful physical examination to exclude any other concurrent illness.

Daily rectal swabs were obtained and cultured on S.S., MacConkey and Monsur's agar plates. Antibiotics were withheld from all patients unless otherwise contraindicated. An acute phase venous blood sample was collected for serological examination, serum solid estimation, and for haematocrit, total white cell and differential counts. Stool samples were examined for faecal leucocytes and parasites.

All patients were kept in hospital for 6 days or until the diarrhoea subsided. All patients were brought back to hospital on 10th day and questioned for subsequent history of loose motion, a rectal swab collected, and convalascent phase venous blood sample was collected to repeat all examination done during hospitalization. If any rectal swab culture taken on admission day was found positive for either cholera, salmonella or shigella by 2nd or 3rd day, the case was dropped from study and appropriate treatment was undertaken. The family contacts were visited on 2nd, 4th and 6th days of hospitalization of index case. During these visits rectal swabs were collected from all family members in Carry-Blair transport medium, and a questionnaire filled up for presence of diarrhoea among family members and their food and water consumption history during previous 48 hours. Control families, drawn from next closest cooking pot family using same water source, were also visited at the same time of visit of index case families and rectal swabs collected from them in each visit and same questionnaire filled up for them. A blood sample was also drawn from one member of control family who is nearest identical age and sex as that of index case for serology. Water samples were also collected from water sources on first family visit for culture.

Bacteriologically 10 E.coli colonies were picked up from MacConkey plates and kept on blood agar base slants along with one pooled sample from 1st day rectal swab culture of an index case. In case of subsequent days' rectal swab cultures from index cases along with E.coli isolations from members of contact and control families and water samples, only one pooled sample from 10 E.coli colonies was kept in

blood agar slant bases.

All E.coli isolated and preserved as described above will be assayed for toxigenicity using Adrenal cell and Chinese Hamster Ovary tissue culture technique. Dr.McLaughlin is identifying toxigenic E.coli strains by CHO technique for index cases and family contacts. Dr. David Sack will be doing adrenal cell assays on all E.coli slants to inadvertently identify toxigenic strains of E.coli and titre, acute and convalascent antibody titres.

Results

A total of 50 cases were studied with 32 males and 18 females ranging in age between 6 months and 65 years. Table 1 shows the age and sex distribution - 56% of cases in <1-3 years age group, 12% in 4-15 years age group and 32% over 15 years age group. Males predominate in first two age groups; while females predominate 15+ age group.

Admission serum solid reports are not available at present, but clinically all cases had mild to moderate degree of dehydration and none was in shock.

Table 2 shows the percent distribution of cases according to days of purging. End of diarrhoea was defined when a patient started passing either soft or formed stool or had no stool for three consequetive eight hour ouput periods. By this definition diarrhoea ended between 2nd and 4th hospital day in 66% of cases. Diarrhoea ended on day of admission in 8% of cases. 12% of cases had their diarrhoea ended by 5th and 6th days. There is another group of 12% of cases in whom diarrhoea continued upto 7th to 9th days. This group represents 6 cases out of 15 cases who developed cholera during their hospital stay. Only 2% of cases representing one case in the series diarrhoea continued for more than 10 days.

Table 3 shows the total stool volume by age groups. It can be seen that "culture negative" cases has a very low mean for stool volume in all age groups. On the other hand the cases who developed cholera (30% of total cases) later had much more stool volume than those who remained "culture negative" throughout their whole hospital course. It may be

recalled here that among 15 cases who acquired cholera only 6 had diarrhoea continued upto 7th to 9th day, while rest 9 had their diarrhoea over by 6th day including one case who was discharged on 6th day but his 10th day rectal swab was positive for cholera without any diarrhoea.

From Table 4 it can seen that there were nine, one and five cholera cases in <1-3 yrs., 4-15 yrs. and over 15 yrs. age groups respectively with males predominating in lower age groups and females predominating in older age group. Diarrhoeal course of these acquired cholera cases is shown in Table 5. In 20% of these cases diarrhoea ended by 2nd day while in 40% of cases diarrhoea continued upto 6th and 9th day.

Table 6 is a detailed analysis of diarrhoea of these cases before and after acquiring cholera. It may be noted that in seven cases (about 47%) diarrhoea ended before acquiring cholera and had no diarrhoea afterwards. Only in three cases (about 20%) diarrhoea restarted after acquiring cholera while in 5 cases (about 33%) had their "culture negative" diarrhoea merged with cholera diarrhoea. It may be mentioned here that only in five of these cases antibiotics had to be used to control diarrhoea after acquiring cholera. It may also be recalled here that the single case in 4-15 year age group had his culture positive for cholera on 10th day when he was brought back to hospital as per protocol after his discharge from hospital on 6th day and he had no diarrhoea at all.

Table 7(a) and 7(b) analyses the incidence of intestinal parasites among the study cases. 38 cases were examined for intestinal parasites. 95% among <1-3 yrs. age group had no parasites in them, while 100% and 93% among two older age groups had one or more parasites in them (Table 7(a)). All the 20 cases in <1-3 yrs. age group had an abundance of neutralfat in stool.

From Table 7(b) we find that there were 3 cases with diarrhoea producing parasites like, E. histolytica, Giardia lamblia trophozoites and larva of Strongyloides stercoralis. In all three of them diarrhoea ended by 2nd day with very little total stool output (200-400 ml) and all of them were in over 15 years age group. Two cases subsequently acquired cholera on 4th hospital day and only one of the two, the one with E. histolytica, had reappearance of diarrhoea after

acquiring cholera with a total output of stool of 30.60 liters and diarrhoea continued upto 7th hospital day. No anti-parasitic medication was administered for any case.

Apart from E.coli isolations, few cholera and shigella were also isolated from contact and control family members. These are shown in table 8(a). It can be seen that while shigella isolations greatly exceed, cholera isolations (48:5), they are almost evenly distributed among contact and control families; but cholera isolations are limited to contact families only.

Bacteriological reports of water cultures from environmental water sources of contact and control families, are shown in table 8(b). With the exception of municipal supply almost all water sources contained E.coli, including one tubewell. Ditch water was uniformly positive. Tanks and canals were positive 90% of the time while 33% of river water were qualitatively positive. These results, seems to be identical with what is expected from the particular water source, except the tubewell water.

Hematocrit, total white cell and differential count values were unrevealing in both acute and convalascent phase samples. The only changes noted were due to dehydration.

Discussion

Clinically all the cases taken in this study had mild to moderate degree of dehydration. Though acute phase serum solid reports are not available at present, 44% of cases first reported to our observation room where we keep patients with no or doubtful dehydration for observation. None of the rest of 56% of cases who were admitted directly was in shock; moreover 60% of cases had history of loose motion for 2-4 days before seeking hospital advice, particularly in < 1-3 yrs. age group. None had fever; few complained of vague abdominal pain with no particular location, which did not require any particular attention or medication. Intestinal parasites isolated from stool might possibly be responsible for such abdominal pains. Over 50% of cases had mucus in their stool, again mostly in < 1-3 yrs. age group. In this age group stools often had a greenish yellow colour and had an abundance of neutral fat. None had bloody stools or faecal leucocytes. The hospital

course was mostly uneventful except in few cases who developed cholera or who had to stay in hospital for more than 6 days for diarrhoea. These cases needed more attention for their repeated needs of hydration. Diarrhoea ended in 74% of cases by 4th hospital day without any need for medication. Stool volume and requirements for fluids were also much less as was in comparison to more severe diarrhoea cases. All these considerations lead one to assume that "culture negative" diarrhoea is relatively mild.

Since the diagnosis of Toxigenic of E.coli, isolated from the study cases, their family contacts and controls and water sources, are pending, there is no scope to limit these data to toxigenic E.coli. Previous investigation of cases at Matlab conforming to these patient selection criteria suggest a sizable proportion of these cases will, in fact, be E.coli diarrhoea. Apart from the fact that 30% of the study cases acquired cholera during their stay in hospital, which will be discussed presently, we know of no other pathogenic organism which could have been responsible for the diarrhoea in them.

Among intestinal parasites isolated, in only three cases were parasites isolated which are known to cause diarrhoea in man, though other parasites might become additional factors for altered bowel behaviors. But even in these three cases diarrhoea was minimum, which ended by second hospital day.

As a rule all patients were required to have at least two successive rectal swabs negative for enteric pathogens before they were formally admitted to the study. Fifteen cases (30%) subsequently developed rectal swabs positive for cholera between 3rd to 8th hospital day. Epidemiological investigations were conducted on four cases comprising two adult females and two male babies.

The two adult females revealed on intense questioning that they both bathed and swam in the canal in front of the Matlab hospital and both drank some of the canal water. Both denied of taking any food brought by their relatives. Environmental swabbing was performed on all things with which the patients might have come in contact including oral solution food and all hospital personnel. All these cultures were negetive for cholera. All family contacts were also

negative for cholera. Except that one of the two male babies had been cared for in his mother's absence by one of the two females discussed above, no other source of infection could be ascertained for the babies. Family contacts and environmental swabbing were negetive in both cases.

It is difficult to make any conclusive statement about the source of infection of cholera in these fifteen patients. Most of them had fully recovered by the third and 4th day of their hospitalization from the "culture negative" diarrhoea, and were moving freely about the hospital environs. Canal water in front of Matlab hospital might have played an important role in transmitting the infection, as it is well established that the canal water is heavily contaminated with vibrios. It is unlikely that outside introduction played any role since all family contacts were culture negative. That person-to-person spread was an important mode of transmission is doubtful as attack rate did not change after all "culture negative" cases were isolated in a separate room during the period of hospitalization.

In all liklihood probably multiple modes of transmission were responsible. There is also a possibility that altered small bowel in E.coli diarrhoea is more susceptible for acquisition of cholera. Such phenomenon was probably responsible in case of only American, Dr. Ely Abrutyn, who acquired cholera at Matlab while he was convalascing from a bout of "Matlab diarrhoea", which occurs to all Americans when they first come in contact with Matlab environment for an extended period of time. No one recalls the acquisition of cholera among patient attendants who are presumably healthy and use the contaminated canal water extensively.

Comment

Pending the final diagnosis of toxigenic E.coli, it can be assumed that mild diarrhoeas for which no known enteric pathogens can be held responsible, might be due to, in some percentage of these cases, toxigenic E.coli. In that event, the illness usually is of short duration, sometimes necessitating intravenous or oral hydration in small quantity. No comment at present can be made about its epidemiological characteristics.

This study was done under sub-optimal circumstances during later part of a cholera epidemic and is meant to demonstrate the scope for improvisation and new clinical enquiry of diarrhoeal disease which exist at Matlab. We propose to repeat the study in the light of experience gained from present study during a non-cholera season.

TABLE - I

AGE AND SEX DISTRIBUTION

Age group	Male	Female	Total	%
1-3	20	8	28	56
4-15	6	0	6	12
15+	6	10	16	32
TOTAL	32	18	50	

TABLE - II

PERCENT DISTRIBUTION OF CASES ACCORDING TO DAYS OF PURGING

No. of days	%	
1	8	
2	22	66%
3	18	
4	26	
5	8	
6	4	
7	6	12% All developed cholera
8	4	
9	2	
10+	2	

STOOL VOLUME IN LITRES

0 4 8 12 16 20

< 1 - 3 years

All Case Combined

Culture Negative and Subsequent Cholera

Only Culture Negative

4 - 15 years

All Case Combined

Culture Negative and Subsequent Cholera

Only Culture Negative

Over 15 years

All Case Combined

Culture Negative and Subsequent Cholera

Only Culture Negative

STOOL VOLUME BY AGE-GROUP AND CULTURE RESULTS

(Mean ± Std. Error of Mean)

TABLE - IV

AGE AND SEX DISTRIBUTION OF CASES WHO DEVELOPED CHOLERA

Age group	MALE		FEMALE		MALE & FEMALE	
	Total case	No. of case dev.cholera	Total case	No. of case dev.cholera	Total case	No. of case dev.cholera
1-3	20	7	8	2	28	9
4-15	6	1	0	0	6	1
15+	6	0	10	5	16	5
TOTAL	32	8	18	7	50	15

TABLE - V

PERCENT DISTRIBUTION OF CHOLERA CASES ACCORDING TO DAYS OF PURGING

Range (days)	% Total cases	% Total cholera
1-2	6	20
3-6	12	40
7-9	12	40
TOTAL	30	100

TABLE - VI

"CULTURE-NEGATIVE" DIARRHOEA:

ANALYSIS OF DIARRHOEA OF CASES BEFORE AND AFTER ACQUIRING CHOLERA

	NO SUBSEQUENT DIARRHOEA			SUBSEQUENT DIARRHOEA							
	E. D. before cholera			E. D. before cholera and diarrhoea restarted afterwards				Diarrhoea continuous E. D. only after cholera			Antibiotics used
Age group	No. of cases	Duration of initial diarrhoea	Stool range ml	No. of cases	Duration of initial diarrhoea	Duration of cholera diarrhoea	Stool range ml	No. of cases	Overall duration of diarrhea	Stool range ml	
1-3	4	3-5	700-2360	0	0	0	0	5	7-9	2290 - 11270	2
4-15	1	4	1,050	0	0	0	0	0	0	0	0
15+	2	2-3	200-4400	3	1-3	7-8	11,500 - 30,600	0	0	0	3

TABLE - VII (A)

AGE DISTRIBUTION OF CASES WITH STOOL POSITIVE FOR INTESTINAL PARASITES

Age group	No. of positive	No. of negative	% Positive	% Negative	Cases not examined
1-3	1	19	5	95	8
4-15	4	0	100	0	2
15+	13	1	93	7	2
TOTAL	18	20	-	-	12

TABLE - VII (B)

INCIDENCE OF PARASITES

Parasites	Single	In combination	Total
Ascaris	4	9	13
Hookworm	0	6	6
Trichuris	2	6	8
Trichomonas	0	2	2
E.Histolytica	1	0	1
Giardia	0	1	1
Strongyloides	0	1	1

TABLE - VIII (A)

CHOLERA AND SHIGELLA ISOLATION FROM STUDY AND CONTROL FAMILIES

Family	Cholera	Shigella	Total
Study (No.50)	5	26	31
Control (No.50)	0	22	22
TOTAL	5	48	53

N.B. Number of members in study and control families were almost identical and so was number of cultures done.

TABLE - VIII (B)

E.COLI ISOLATION FROM DIFFERENT WATER SOURCES OF STUDY AND CONTROL FAMILIES

Source	No. sampled	% E.coli isolation
Tubewell	13	7
Municipal supply	1	0
Ditch	8	100
Tank	41	90
Canal	6	90
River	6	33
TOTAL	75	

PROCEEDINGS OF THE 9TH MEETING OF THE SCIENTIFIC REVIEW AND TECHNICAL ADVISORY COMMITTEE OF THE CHOLERA RESEARCH LABORATORY

(a)

and

REPORTS OF THE COLLABORATIVE STUDIES BETWEEN CENTER FOR MEDICAL RESEARCH AND CHOLERA RESEARCH LABORATORY

For the

YEAR 1974

Dacca, Bangladesh