Etiology of Diarrhoea Among Adult Patients During the Early Monsoon Period in Kathmandu, Nepal

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Abstract: One hundred and eighty-one patients with acute diarrhoea attending the gastroenteritis ward of Sukra Raj Tropical and Infectious Disease Hospital (STIDH) were investigated during the early monsoon, April to May 2001. Bacterial pathogens were isolated in 33% of the patients. Enteropathogenic *Escherichia coli* was isolated in 8.28%, *Shigella* species in 13.25% and *Vibrio cholerae* 01 in 1.1% of the patients. Mixed infections with bacterial pathogens, helminths and protozoan parasites were commonly observed in the study. *Trichuris trichiuria* was detected in 27.6%, hookworms in 12.7% and *Ascaris lumbricoides* in 11.04%. *Entamoeba histolytica/dispar* and *Giardia lamblia* were observed in 12.7% and 7.73% of the patients, respectively. A large number of *Cryptosporidium* (7.73%) and *Cyclospora* species (3.86%) usually present in immunocompromised patients were also detected in acute diarrhoeal cases. The results showed that a wide range of bacterial pathogens was isolated from the inhabitants of Kathmandu, Nepal prior to the monsoon. These findings indicate that the bacterial pathogens, especially diarrhoeaginic *E. coli* and *Shigella*, and protozoan parasites, need to be given additional attention in the diagnosis and treatment of acute diarrhoea.

Key words: Acute diarrhoea, etiology, bacterial pathogens, helminthic parasite, Nepal

INTRODUCTION

Diarrhoea is the most common illness among children in the developing world. It is the primary cause of mortality and morbidity and the highest risk occurring within the first 5 years of life (Bern *et al.*, 1992). However, it is also a serious public health problem among adults, causing significant levels of morbidity during early summer and the rainy season in developing countries like Nepal (WHO, 1992; Bern *et al.*, 1992). The incidence of diarrhoeal diseases in Nepal tends to rise sharply during warm summer months with the peak incidence occurring in July and August. Food borne gastroenteritis does increase in April and May each year, followed by water-borne gastroenteritis and cholera in the beginning of rainy season (WHO, 1992). Sukraraj Tropical and Infectious Disease Hospital (STIDH) is the only infectious disease hospital receiving adult patients with diarrhoea in Kathmandu. A total of 4497 patients with diarrhoea were admitted in the year 2000 in STIDH. Although several studies have been conducted in the past, there are no clear figures on the etiology of diarrhoea in the adult population (WHO, 1992; Hoge *et al.*, 1995; Sherchand and Shrestha, 1996). Despite a number of individual hospitals setting up their own guidelines at present, there is still no established national protocol for the management of diarrhoeal diseases. In this situation, etiological study of diarrhoeal disease is very important for the provision of base-line information on the causes of diarrhoeal disease and preparation of a na-

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tional protocol for the management of diarrhoea.

The present study was initiated to establish the etiological agent responsible for acute diarrhoea in the adult population with samples collected from inhabitants of the Kathmandu Valley.

Materials And Methods

Patients attending the STIDH those with acute diarrhoea were enrolled in the study. Criteria for the exclusion from the study included patient's administration of antibiotics and systemic illness (heart disease, enteric fever, leshmaniasis, central nervous system manifestations). The hospital staff completed a set of questionnaires after obtaining written consent from the patients. Demographic profile was recorded, general physical examination was performed and severity of dehydration was assessed. Stool samples were collected, and appropriate treatment was started based on the degree of dehydration. Samples were subjected to routine parasitological and bacteriological examination. Treatment was reviewed after receiving the laboratory reports for the stool test.

Parasitological test

The stool samples were collected in the morning from 181 patients older than 14 years in STIDH and were examined. Direct smears were prepared with normal saline, iodine, and sucrose solution. Two and half percent of potassium dichromate solution and modified Ziehl Nelsen staining were applied to differentiate protozoan parasites into mainly *Cryptosporidium*, *Isospora* and *Cyclospora* (Casemore, 1991). A formal ether concentration method was also used to identify other intestinal parasites.

Bacteriological test

Samples received in the laboratory were cultured immediately to identify pathogenic microorganism such as *Vibrio cholerae, Salmonella* species, *Shigella* Species and *Escherichia coli* using standard bacteriological method (Mackie and McCartney, 1996). Stool samples were inoculated onto thiosulphate citrate bile salt sucrose (TCBS) Agar, MacConkey agar, eosin methylene blue (EMB) agar as well as SS agar, and into enrichment media such as Selenite F, Rappaport and Alkaline peptone water (pH 8.5). After 24 hours of incubation at 37℃, Rappaports and Selenite F broth cultures were inoculated onto MacConkey and SS agar, respectively. Alkaline peptone water was subcultured onto TCBS agar after 12 hours and incubated overnight at 37℃. Typical characteristic vibrio like colonies seen on TCBS agar were subcultured onto nutrient agar and identified by biochemical test. Biotypes of *V. cholerae* were determined using Voges-Proskauer (VP), polymyxin B sensitive and haemolysin production test (Blood agar). Biochemically confirmed *V. cholerae* was subjected for serotyping. Serotyping was carried out with the use of diagnostic antiserum raised against *V. cholerae* manufactured by Denka Seiken, Japan as per manufacturer's protocol.

Both lactose and non-lactose fermenting colonies from MacConkey and SS Agar were subjected to further identification. Biochemical and serological tests such as TSI, SIM, VP, dycarboxylase, simons citrate medium. Diagnostic antiserum (Denka Seiken Co. LTD Tokyo, Japan) was also used for serotyping of enteropathogenic *E. coli*, *Salmonella* and *Shigella* species. Skirro's medium was used for the isolation of *C. jejuni* and suspected colinies were further examined by gram stain. The strains identified as above were also confirmed by using API 20E *in vitro* diagnostic biochemical kit manufactured by bioMerieux SA 69280 Marcy/Etoile-France.

Statistical methods

Differences between valuse were examined using Student's t-test analysis was carried out using SPSS version 10, Statistical Analysis Software, 2000.

Results

A total of 181 patients aged 14 years and above attending the STIDH between April and May with acute diarrhoea was included in this study (Table 1). The patients recorded, an average, 10-15 loose motions before coming to the hospital. The patients reported an average of 11 hours of loose motion before coming to the hospital. Of those 60% of the cases were female (P<.0001) and the highest numbers (40%) were in the age group between 20-29 for both males and females (Fig. 1). The main symptoms associated with diarrhoea were combination of abdominal pain (70%), vomiting (62%), and fever (15%) with moderate to severe dehydration (Fig. 2).

Since the etiology of diarrhoea has not been studied in adult population, the prevalence of all the enteropathogens including *Cryptosporidium* and *Cyclospora* was also determined. Bacterial pathogens were isolated from 33% of the patients (Table 1). Among them enteropathogenic *E. coli* (EPEC) were isolated from 8.28%, *Shigella* species from 13.25% (*Shigella dysenteriae* in 5%, *S. flexneri* in 4.41% *S. boydi* in 2.2% *S.sonnie* in 1.65%). The result also showed that EPEC was the most commonly isolated single bacterial pathogen, followed by *S. dysenteriae* 5% and *Salmonella* species was isolated in 4.41% of the cases tested.

The result showed that 40% of the patients were infected with more than one organism. Protozoal pathogens

Table. 1 Enteric pathogens identified among 181 patients of diarrhoea from April and May, 2001 in STIDH, Teku, Kathmandu

Serial Number	Enteropathogens	Patients (n＝181)	Percentage
1	Enteropathogenic *Escherichia coli*	15	8.28
2	Enterotoxigenic *Escherichia coli*	5	2.76
3	Enteroinvasive *Escherichia coli*	4	2.20
4	Enterohaemorrhagic *Escherichia coli*	2	1.10
5	*Shigella dysenteriae*	9	5.0
6	*Shigella boydi*	4	2.2
7	*Shigella flexneri*	8	4.41
8	*Shigella sonnei*	3	1.65
9	*Salmonella species*	8	4.41
10	*Vibrio cholerae* 01	2	1.1
11	*Camphylobacter jejuni*	1	0.55
12	*Entamoeba histolytica/ E. dispar*	23	12.7
13	*Cyclospora species*	7	3.86
14	*Cryptosporidium*	14	7.73
15	*Blastocystis hominis*	14	7.73
16	*Ascaris lumbricoides*	20	11.04
17	*Giardia lamblia*	14	7.73
18	*Trichuris trichiuria*	50	27.6
19	Hookworm	23	12.7
20	*Hymenolepsis nana*	2	1.1
21	*Chilomastix mesnili*	3	1.65
22	*Dientamoeba fragilis*	1	0.55
23	Trichomonas	1	0.55
24	Strongyloides	3	1.65
25	*Isospora belli*	1	0.55
26	*Iodamoeba butschlii*	3	1.65

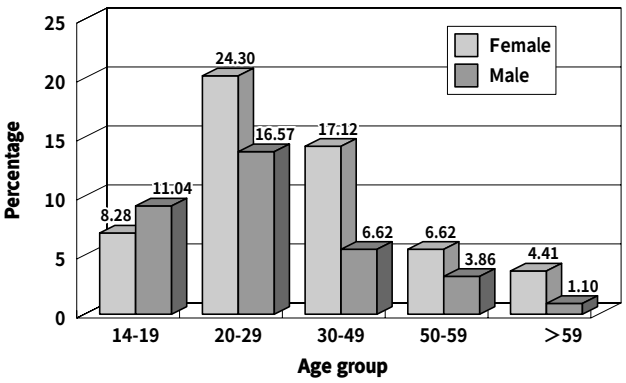

Fig.1: Percentage of sex and age distribution of patients with acute diarrhoea.

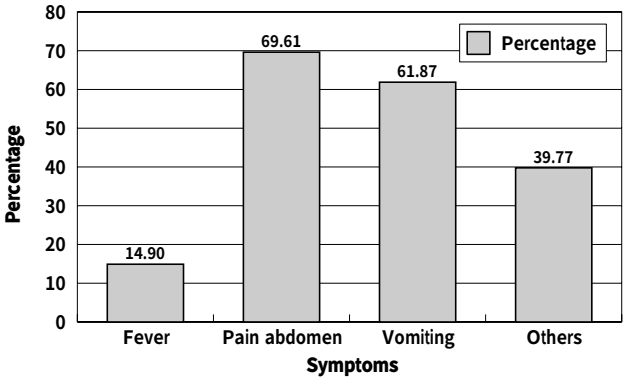

Fig.2: Percentage of other symptoms associated with diarrhoea on admission to hospital in Kathmandu, Nepal, 2001.

E. histolytica/E. dispar, G. lamblia, Cryptospridium and *Cyclospora* were detected in 31% of the patients. Of these 12.7% were infected with *E. histolytica/E. dispar*, 3.86% with *Cyclospora* and 7.73% each with *G. lamblia* and *Cryptosporidium*. It was also observed that *T. trichiuria* infection was the predominant helminthic infection (27.6%) among the patients. On the other hand, 13% of the patients were infected with hookworm and 11.04% with *A. lumbricoides*. The symptoms associated with *Blastocystis hominis* are similar to those associated with *G. lamblia* both of them isolated in 7.73% of the patients. *Hymenolepsis nana* and *Isospera belli* were detected in 1.1 and 0.55% of the patients, respectively.

Although *V. cholerae* 01 is the major cause of acute watery diarrhoea during the rainy season, it was only isolated 1.1% in our study period, which was conducted during early part of the rainy season. On the other hand rare pathogens like *Chilomastix mesnili, Iodamoeba butschlii* were also detected in the range of 1% and *Strongyloides* was detected in 1.65% of the cases.

DISCUSSION

This paper is one of the first studies to examine the etiology of diarrhoea during warm early monsoon among the adult Nepalese population. This study confirms the importance of *E. coli* species, *Shigella species, E. histoytica, G. lamblia* and *Cryptosporidium* as the causative agents of acute diarrhoea in Nepal. Hoge *et al.* (1995) reported similar observation among children under five years. Our results indicate that *Cryptosporidium* and *Cyclospora* species are also important pathogens in Kathmandu. The low prevalence of cholera in this study is most likely due to the season during which our sampling was undertaken. It is important that an etiological study should be continued for a complete year irrespective of age of the patients.

Diarrhoea is usually classified as either inflammatory or non-inflammatory disease since such a classification has therapeutic significance. The other categories of diarrhoegenic *E. coli*, (not isolated in this study) like enteroaggregative *E. coli*, have also been shown to be an important causal agents of acute diarrhoea in developing countries (Bouckenooghe *et al.*, 2000). More extensive study is required to establish their role in the Nepalese context. Inflammatory diarrhoeas that may be associated with higher mortality can be studied by stool culture. Several inflammatory markers like faecal leucocytes, occult blood, and fecal lactoferrin could be used as a predictor of invasive pathogen in enteric inflammation (Huicho *et al.*, 1996).

The source of drinking water, knowledge and attitude of the patients and the family members were assessed in this study. As shown in Fig. 3, 80% of the patients reported to use their tap water as a source of drinking water, has low chlorine concentration and which is polluted with high numbers of coliform in Kathmandu (WHO, 1992). Only 23% family's boil their drinking water and the rest of the people drink water without boiling which could be one of the major predisposing factors for diarrhoea. We attempted to access the knowledge regarding the etiology of diarrhoea during the study. Forty-five percent of the patients still believe that diarrhoeal diseases are not communicable. Contaminated public water supplies probably contributed significantly to sustained transmission of disease in the Kathmandu Valley. Health education and improvement of sanitation are necessary for prevention. Increased administration of oral rehydration solutions is vital in order to decrease the morbidity and mortality from acute diarrhoea. The present study indicates that there is a seasonal variation on the etiology of diarrhoea in Kathmandu Valley, which should be confirmed by more extensive longitudinal study.

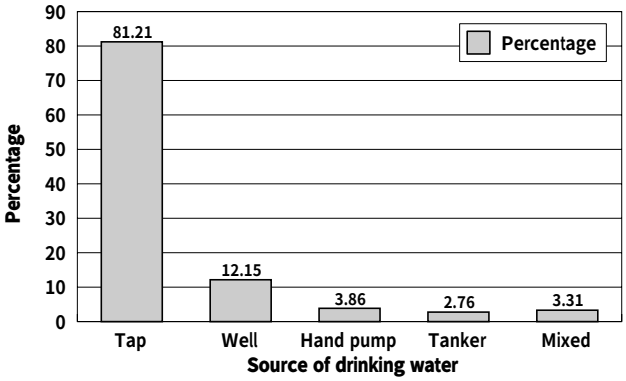

Fig.3: Source of drinking water in the inhabitants of Kathmandu, Nepal

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