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Full Length Research Paper

The frequency of *Escherichia coli* (EPEC, ETEC, EIEC and serotypes) shigella, rotavirus and parasite agents among children with acute gastroenteritis in Southeast Anatolia, Turkey

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This study analyzed a host of parasite agents and bacterial viruses in 1,079 children aged 0 to 5 years suffering from diarrhea between 2004 and 2008. Enteric pathogens were detected in 767 (71%) infected patients and 456 control patients. Enteric pathogens were isolated from 41 (8.9%) of the infected patients. Isolated pathogens responsible for gastroenteritis were as follows: Rotavirus 282 (26.1%), Shigella 19 (1.7%), enteropathogenic *Escherichia coli* 153 (60.9%), entero-invasive *E. coli* 30 (11.9%) and enterotoxigenic *E. coli* 68 (27%). Among the *E. coli* serotypes; O111:K58:B4, 055:K59:B5, 0127:K63:B8:, 026:K60:B17, 124:K72:B17, 0125:K70:B15 were found in 48, 39, 29, 19, 9 and 5 (19.1, 15.5, 11.5, 7.5, 3.5 and 1.9%) of the cases, respectively. In two of the infected patients, both 128:K67:B12 and 0112:K66:B11 types were isolated together. Also, 0112:K66:B11 and 0128:K67:B12 types were isolated together only in two cases. Rotavirus was detected by enzyme immunoassay (EI) and rapid latex agglutination (LA) test in 26.1% of the fecal specimens from 1,079 children who had acute diarrhea and 4.3% of the fecal specimens of the 250 healthy controls. Infections were detected most commonly in children aged 0 to 2 months (54.8%). In the identification of rotavirus, the two methods, LA and EI were also compared. In diarrheal fecal samples, *Entamoebae histolytica* was found in 25.4% and *Giardia lamblia* was found in 18.4% patients.

Key words: Gastroenteritis, enteropathogens, rotavirus.

INTRODUCTION

Diarrheal diseases are major causes of illness and death among infants and young children, especially in developing countries. Nosocomial diarrhea is an important cause of childhood morbidity and mortality. Modifications of hygiene procedures and preventive measures are necessary in order to reduce nosocomial infections. Nosocomial diarrhea is also caused by bacterial pathogens such as Campylobacter, enteropathogenic and enterotoxigenic *E. coli* and Shigella. They are the most common bacterial enteropathogens isolated in developing countries. Campylobacter and Shigella are more prevalent in developed countries (Abdulla et al., 1999). Diarrheal illnesses in children younger than 5 years is one of the leading causes of morbidity and mortality in developing countries and is an important cause of morbidity in developed countries. Children younger than 5 years in developing countries have approximately 3 to 9 diarrheal illnesses per year, whereas in North America young

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children have an average two diarrheal episodes per year. In the United States, children younger than 5 years have 37 million episodes of diarrhea annually (Albert et al.,1995; Bryce et al., 2005).

Acute diarrhoea is one of the leading causes of morbidity and mortality in children in most developing countries. Despite the use of oral rehydration therapies, deaths due to diarrhoea in children aged less than five years are still estimated to be about two million per year (Mandomando et al., 2007).

Diarrhea is recognized as an important public health problem among children in developing countries and is a research priority of the Diarrheal Diseases Control Program of the World Health Organization in 1976. John Rohde who highlighted the importance of diarrhea as a prime killer of children in the developing world, asked the scientific community to "take science where the diarrhea is". The World Health Organization estimates that 1 billion diarrheal episodes occur worldwide in infants annually resulting in 3.3 million deaths, making diarrheal disease a major contributor to infant mortality in the developing world. The need for simple, effective and inexpensive intervention to treat diarrhea and prevent its occurrence is urgent and abundantly clear (Abdulla et al., 1999). In developing countries, it has been estimated that more than 870,000 children die from rotavirus infection every year (Abdulla et al.,1999; Albert et al.,1995). Our aim is to bring into consideration the incidence of enteropathogens among children in such poor living conditions of the area.

MATERIALS AND METHODS

This study analyzed a host of parasite agents and bacterial viruses in 1,079 children aged 0 to 5 years, suffering from diarrhea between 2004 and 2008. Obtained stool samples were transported in stool cups to the laboratory for microbiological examination. Fresh stools were cultured by using standard methods. Fresh stools were also examined for parasites. Formal-Ether methods were used for detecting responsible enteropathogens.

Specimen collection and processing

Stool swabs were inoculated into MacConkey and Eosin Methylene Blue agars (Oxoid, England) and streaked for isolated colonies. Specimens are inoculated into transport media (Bioner) and plated out as soon as possible, generally within 24 h. After incubation for 24 and/or 48 h at 37°C, three or four colonies with typical morphology were streaked on fresh plates. Each colony was independently subjected to biochemical tests for the identification of responsible pathogens (Wilke et al., 2002).

Rotavirus enzyme immunoassay (EI) and rapid latex agglutination (LA) (IDEIA, Denmark) were used in rotavirus investigations and both tests were compared to each other. The Virotect-Rota test (Omega, UK) was performed according to the recommendations of the manufacturer. Samples from enteropathogenic *E. coli*, enteroinvasive *E. coli*, and enterotoxigenic *E. coli* were analyzed with the colist enzyme-linked immunosorbent assay (ELISA) kit (Denka Seiken, Tokyo, Japan). To identify serotype O111:K58:B4, O55:K59:B5, 0127:K63:B8, 026:K60:B17, 0124:K72:B:17, 0125:K:70B15, 0112:K66:B11, 0128:K67:B12 and Shigella (polyvalent 1 to 10) (Murex,UK) were used.

Statistical analysis

Statistical analysis was carried out using the SPSS 15.0 program (SPSS 15.0 for Windows Evaluation Version Release 15.0; 6 September 2006). The chi-square test was used in the evaluation of different variables. P = 0.05 was considered to be statistically significant.

RESULTS

1,079 faecal samples were collected from children (0 to 5 years) suffering from acute diarrhea in Diyarbakır, Turkey. One or more enteropathogens were identified in 923 (85.5%) of the stool specimens. Mixed infections were detected in 156 (14.4%) diarrheal cases. Only 767 (71%) patients were infected with a single pathogen. Rotavirus was detected in 93 (32.9%) of the specimens of hospitalized patients.

Other responsible agents included *E. coli* 251 (23.2%), of which 153 (60.9%) were enteropathogenic *E. coli* (EPEC), 68 (27%) enterotoxigenic *E. coli* (ETEC) and 30 (11.9%) enteroinvasive *E. coli* (EIEC). Among *E. coli* serotypes, O111:K58:B4, 055:K59:B5, 0127:K63:B8:, 026:K60:B17, 124:K72:B17, 0125:K70:B15 were found as 19.1, 15.5, 11.5, 7.5, 3.5 and 1.9% of the cases, respectively. Togetherness of the serotypes of 128:K67:B12 and 0112:K66:B11 were found only in two cases (0.7%). Other isolated pathogens were *Giardia lamblia* 198 (18.4%), Shigella 19 (1.7%), *Entamoeba histolytica* 274 (25.4%) and *Candida albicans* 55 (5.1%). The prevalence of isolated enteropathogens were given in Table 1 and 2.

A comparison of the results obtained with the Virotect-Rota latex and Rota EI tests among different age groups were shown in Table 3. Of the 1,079 study specimens, 282 (26.1%) and 270 (25%) yielded positive results with the Virotect-Rota latex test and Rota EI test, respectively. Twelve positive Rota EI specimens were negative in the Virotect-Rota latex test. The difference was not significant ($p > 0.05$).

Mixed infections were relatively frequent with the following distribution: *E. coli* (EPEC, ETEC, EIEC) and rotavirus were found responsible from gastroenteritis in 28 cases, whereas rotavirus and *G. lamblia* mixed infections was found in 9 cases; Rotavirus and *E. histolytica* in 13 cases, *E. coli* (EPEC, ETEC, EIEC) and *C. albicans* in 38 cases, *E. coli* and *G. lamblia* in 32 and *E. coli* and *E. histolytica* in 36 cases.

DISCUSSION

Diarrhea is the one of the leading cause of death related to infectious diseases all over the world; the rate of death due to diarrheal diseases is estimated as two million per

Table 1. Identification of enteric in children with diarrhea 1079 faecal sample.

Enteropathogens	Number	%
Escherichia coli	251	23.2
Rotavirus	282	26.1
Entamoeba hystolytica	274	25.3
Giardia lamblia	198	18.3
Candida albicans	55	5.0
Shigella	19	1.7

Table 2. The ratio of *Escherichia coli* and serotypes in childeren with diarrhea 1079 faecal sample *E. coli* 251 (23.2).

***Escherichia coli* n:251 serotype**		Number	%
EPEC		153	60.9
ETEC		68	27
EIEC		30	11.9
Serogroups	0111:K58:B4	48	19.1
	055:K59:B5	39	15.5
	0127:K63:B8	29	11.5
	026:K60:B17	19	7.5
	0124:K72:B17	9	3.5
	0125:K70:B15	5	1.9
	0128:K67:B12	2	0.7
	0112:K56:B11	2	0.7

Table 3. Comparison of the results obtained by virotect-Rota LA and Rota EIA.

N: 282	Virotect-Tota LA		Rota EIA	
	(+) case	%	(+) case	%
0 - 2	148	54.8	154	54.6
3 - 5	122	45.1	128	45.3
0 - 5 (1079)	270	25	282	26.1

year. Gastroenteritis caused by viral agents is gradually increasing particularly in developed countries (Farkas and Jiang, 2007). Although the improvements in sanitation have significantly decreased the gastroenteritis caused by bacteria and parasites, it has little effect on viral gastroenteritis (Farkas and Jiang, 2007) Bacteria and parasites still is an important cause of diarrhea worldwide. The present study was aimed to investigate the prevalence of viral, bacterial and parasitic pathogens that cause infectious diarrhea among children aged less than five years in Diyarbakir, an endemic city in southeastern Turkey. Our results are comparable with the most studies conducted from other parts of the world.

Human rotaviruses are the most important etiologic agents of acquired diarrhea in infants and young children worldwide (Turhanoglu et al., 1991; Raboni et al., 2001). Rotavirus was the most frequent isolated bacterial enteric pathogen and was found in 26.1% of samples.

Gimprel et al. reported the hospitalization rate in high ratios approximately 16.5 to 21%, reaching 22 to 26% at the winter peak. Overall, rotaviruses were identified in 22.4% of stool samples but the detection rate increased from 10% in 1989 to 31% in 1997. Rotaviruses were isolated mainly in winter, reaching the rate of 50 to 70% at the peak (Grimprel et al., 2001).

The present study clearly shows that rotavirus is the most common cause of acute gastroenteritis among admitted children (26.1%) in Diyarbakır, Turkey. A research conducted in France detected the Rotavirus rate as 17.3% (Marie-Cardine et al., 2002). Huh et al. (2009) collected 10,028 stool samples from all age groups and found the rotavirus frequency of 19.3% among all viral agents. This rate is very close to the rotavirus positivity that we have determined in our study. Although rotavirus gastroenteritis is more common in children under 5 years of age, it can be seen with a definite frequency in every age group (Huh et al., 2009). In a study, it was reported that rotavirus was found in 56% of 451 hospitalized gastroenteric children aged 4 to 36 months age (Chowdhury et al., 2001), whereas in another study rotavirus was found in 40.2% of 2,319 fecal specimens from gastroenteritis in children (O'Mahony et al., 2000).

In a study, 80% of diarrheal cases were observed under 2 years of age and rotavirus (19.2%) was found the most frequent enteropathogen associated with diarrhea, followed by *Shigella flexneri* (6.15%) and *Shigella sonnei* (1.5%) (Orlandi et al., 2001). Rotavirus was detected the frequent agent among 6 to 11 month-old children, accounting for 20% of all cases in this age group (Vargas et al., 2004). These results are quite similar to those found in our study.

Collected fecal specimens from 3,177 children with acute diarrhea in ten regions of China between April 1998 and April 2000 were screened for rotavirus; and the rate was found as 41% (1,305) of specimens (Fang et al., 2002). Furthermore, the studies in the literature have also focused on the pediatric age group due to the high incidence of morbidity and mortality risk in this age group. Regarding these data, although rotavirus gastroenteritis is more common among children under five years of age, it can be seen with a definite frequency in every age group.

In the trials carried out in different countries, rotavirus positivity varies between 20 and 50% in different pediatric age groups (Akan et al., 2009; Bicer et al., 2006; Gul et al., 2005; Turhanoglu et al., 1991).

LA assays for rotavirus diagnosis are rapid and inexpensive, and are the most widely used screen specimens. The performance of the LA Rotagen (Omega) was evaluated for rotavirus detection in fecal samples of outpatients and those hospitalized with acute gastroenteritis. This assay was compared with EI. Rota

EI gave 282 positives and Rota LA provided 270 positives for rotavirus. The difference was found statistically insignificant. Rota-virotect LA is cheaper and most practical technique using in the identification of rotavirus. Almost all studies conducted worldwide seem to conclude that the most frequently seen pathogen among diarrheal children is rotavirus infections.

Epidemiological evidence and human challenge studies have demonstrated unequivocally that EPEC, ETEC, EIEC and EHEC are important causes of diarrhea worldwide (O'Mahony et al., 2000). Bacterial enteropathogens were identified in 23.2% of 1,079 diarrheal specimens and these were studied to isolate EPEC, ETEC and EIEC *E. coli*. About 60.9% EPEC, 27% ETEC and 11.9% EIEC were isolated from diarrheal patients. Among the 12 different serotypes of EPEC isolated, 0111:K58:B4(19.1%) was predominant. Schultsz et al. (2000) detected *E. coli* in 18 (10.7%) of 169 patients and 4 (3.7%) of 108 controls in stool samples.

Okeke et al. (2000) isolated the subtypes of *E. coli* as enteropathogenic *E. coli* (1.8%), enterotoxigenic *E. coli* (2.4%), enteroinvasive *E. coli* (1.2%) and enterohemorrhagic *E. coli* (0.6%). In a study conducted in Saudi Arabia, 249 faecal samples of the children under three years were analysed and 60 (24%) *E. coli* were isolated (El-Sheikh and El-Assovli, 2001). In Mirzapur, 1351 faecal samples of the children younger than three years of age were analyzed and 244 (18%) *E. coli* was isolated (Khundkar et al., 2006). Qadri et al. (2000) noted that enterotoxigenic *E. coli* was detected in 14% of 4662 children with diarrhae.

Orlandi et al. (2001) identified 3.1% ETEC, 2.3% EPEC and 0.8% EIEC in 130 diarrheal patients. In a clinical epidemiological study conducted in Jordanian children under five years of age, Mohammad Yousse et al. Detected enteropathogenic *E. coli* (12.8%), enteroaggregative *E. coli* (10.2), enterotoxigenic *E. coli* (5.7%), (Youssef et al., 2000). In a study conducted in Mirzapur, the frequency of ETEC generally increased with the age of the patients in the following order: <6 months 14.0%; 6 to 11 months 16.2%; 12 to 17 months, 21.7%; 18 to 23 months, 18.4%. (Khundkar et al., 2006).

Rajendran et al. (2010) examined 394 children with diarrhea. Enteroaggregative *E. coli* was found as the most common pathotype followed by enteropathogenic *E. coli* seen in 40 (10%) cases and 32 (8%) of controls. Enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) were found in 16 (4.0%), 8 (2.0%), 4 (1.0%), and 2 (0.5%) of cases, respectively. ETEC was found in 10 (2.5%) of controls, but EHEC, EIEC, and DAEC were not detected.

A study conducted in the Manhica District Hospital, *E. coli* isolated as the most frequently responsible pathogen in children with diarrhea and was followed by *Ascaris lumbricoides* (9.3%) as a second pathogen (Mandomando et al., 2007).

Worldwide, it is estimated that shigellosis causes around 600,000 deaths per year, two thirds of the deceased children is generally under 10 years of age. *Shigella dysenteria* and *S. flexneri* are the predominant species in the tropics, while *S. sonnei* is the predominant species in industrialized countries. In a study, Shigella was isolated from 4.9 and 8.4% of children with gastroenteritis (Youssef et al., 2000; Serichantalergs et al., 2008).

In another study, 134 strains of *Shigella* spp. were subtyped, and subtypes were distributed as follows; 94 (5.37)% *S. flexneri*, 14 (0.80%) *Shigella dysenteriae* and 10 (0.57%) Shigella sonnei from diarrhoeal children (Khundkar et al., 2006). Orlandi et al. (2001) isolated 6.1% *S. flexneri* and 1.5% *S. sonnei* among diarrhoeal children. In Tanzania, the 88 strains of *Shigella spp*. isolated were distributed as follows: 78 (90%) were *S. flexneri*, 4 (4.6%) were *S. dysenteriae*, and 4 (4.6%) were *S. sonnei* (Vargas et al., 2004).

Parasitic agents, especially *E. histolytica* and *G. lamblia*, have also been etiological agents of diarrhea in children. In this study conducted among 0 to 5 year-old children with acute gastroenteritis, parasitic infections ratio were found as 43.7%, of these infections the most isolated parasites were *E. histolytica* (25.4%) and *G. lamblia* (18.4%). Faraj noted that out of 779 cases, 389 (50%) were positive for parasitic infections and the most responsible protozoan parasite was found *E. histolytica* (40.9%) and *G. lamblia* (8.6%) (Faraj, 1999). In another study, Shultsz et al. (2000) noted that *G. lamblia* was identified in 20 of 169 (11.8%) diarrheal patients. Mixed infections were also frequent, associating rotavirus, EPEC and *Salmonella sp*. with *E. histolytica* and *G. lamblia* (Orlandi et al., 2001)

This study concludes that rotavirus is very active in children aged 0 to 2 years. Rotavirus-associated diarrhea is still so common, particularly in the first two years of life. In this article, we reviewed data and current epidemiologic profiles of gastroenteritis in children in an endemic city in the southeastern part of Turkey. We showed that the responsible pathogens are similar in the previous studies done in our region and also we want to emphasize on issues for consideration of required new vaccines and regular strict vaccine proggrammes.

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