Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2017;42(4):617-622
DOI: 10.17826/cutf.325568

ARAŞTIRMA / RESEARCH

Microbiological evaluation of an acute gastroenteritis outbreak

Akut gastroenterit salgınının mikrobiyolojik değerlendirmesi

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Cukurova Medical Journal 2017;42(4):617-622

Abstract

Purpose: Acute gastroenteritis is one of the most important causes of morbidity and mortality especially in children. In water-borne outbreaks, more than one infectious agent may act as the cause of the disease due to contamination of water with feces.

Material and Methods: We evaluated 440 patients who applied to Şırnak State Hospital Emergency Service in two weeks. Their complaints were; vomiting, abdominal pain and diarrhea and were pre-diagnosed as gastroentiritis. Fresh stool samples were examined by native-lugol method for parasitological examination. For bacteriological examination stool samples were inoculated on Salmonella/Shigella agar and EMB agar. For Rotavirus, adenovirus and Entamoeba histolytica detection, rapid diagnostic assays were used.

Results: We detected one or more intestinal parasites in 146 samples (33.2%). We identified 96 Entamoeba histolytica (21.8%) and 44 Blastocystis (10%), and we found Giardia lamblia in 24 (5.5%) of them. 16 (3.6%) of the stool samples were positive for Rotavirus. In any of the samples neither adenovirus nor Salmonella nor Shigella spp. were detected.

Conclusions: The reason for this outbreak may be the pollution of drinking water with sewage water and lack of adequate water purification. In order to prevent such diseases the sterilization of the drinking water appropriately is very crucial.

Key words: Gastroenteritis, outbreak, parasites, rotavirus.

Öz

Amaç: Akut gastroenterit özellikle çocuklarda morbidite ve mortalitenin en önemli nedenlerinden biridir. Su kaynaklı salgınlarda, suyun dışkı ile kirlenmesine bağlı olarak, birden fazla tür etken olarak rol oynayabilmektedir.

Gereç ve Yöntem: Bu çalışmada, iki haftalık süre içerisinde bulantı, kusma, karın ağrısı ve ishal şikayetleri nedeniyle Şırnak Devlet Hastanesi Acil Servisine başvuran 440 hasta akut gastroenterit ön tanısı ile değerlendirmeye alınmıştır. Hastalardan alınan taze dışkı örnekleri parazitolojik inceleme amacıyla nativ-lugol yöntemleriyle incelenmiştir. Bakteriyolojik inceleme için ise dışkı örnekleri Salmonella/Shigella agar ve EMB agara ekilmiştir. Entamoeba histolytica, Rotavirüs ve Adenovirüs tanısı için hızlı tanı kitleri kullanılmıştır.

Bulgular: Örneklerin 146'sında (%33.2) bir veya daha fazla sayıda bağırsak paraziti tespit edilmiştir. Bu örneklerin 96'sında (%21,8) Entamoeba histolytica, 44'ünde (%10) Blastocystis türleri, 24'ünde ise (%5,5) Giardia lamblia saptanmıştır. Dışkı örneklerinin 16'sında (%3.6) Rotavirüs pozitif bulunmuştur. Adenovirüs, Salmonella ve Shigella hiçbir örnekte saptanmamıştır.

Sonuç: Bu salgının sebebinin kanalizasyon atıklarının içme suyu şebekesi ile karışması ve yeterli su arıtımının sağlanamaması olduğu düşünülmüştür. Bu tarz hastalıkların önlenmesinde, içme suyu arıtımının en iyi şekilde yapılması büyük önem taşımaktadır.

Anahtar kelimeler: Gastroenterit, salgın, parazitler, rotavirus.

INTRODUCTION

Acute gastroenteritis (AGE) is severe inflammation of stomach and intestines which is one of the most significant causes of morbidity and mortality. Each year approximately 3-5 billion of acute gastroenteritis case develops around the world and it is estimated that it causes two million deaths annually, particularly in children under the age of five. The disease is usually caused by the

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Geliş tarihi/Received: 15.12.2016 Kabul tarihi/Accepted: 14.03.2017

consumption of water contaminated with feces. The most common symptoms are sudden onset of diarrhea, nausea, vomiting and abdominal pain. While the most common infectious agents are viruses, bacteria and parasites can also cause AGE[1,2].

Rotavirus is the most common viral agent causing AGE among children under five years in both developed and developing countries. Enteric adenovirus, astrovirus, and norovirus are other important viral factors for acute gastroenteritis[3]. Viral gastroenteritis can cause epidemics. Norovirus that belongs to calicivirus family is considered as the responsible agent for AGE outbreaks in all age groups globally[4,5].

The most frequent agents cause of acute bacterial gastroenteritis are Escherichia coli, Shigella, Salmonella, Campylobacter, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Vibrio cholera and Listeria monocytogenes. Bacterial pathogens can lead to outbreaks especially originating from contaminated food and drinking / tap water[6]. Parasites are relatively less common causative agents. The most common parasites that may cause epidemics are Giardia lamblia, Cryptosporidium, Cyclospora cayetanensis and Entamoeba histolytica[7]. The outbreak is defined as the occurrence of cases of a particular disease more than what would normally be expected in a defined area or population and a defined period. In this study, admittance of numerous patients with AGE complaints to the emergency room was considered as an outbreak and microbiologic examinations were done to determine possible causes.

MATERIAL AND METHODS

In this study, we evaluated 440 patients who admitted to Şırnak State Hospital Emergency service between September 1-14 with complaints of nausea, vomiting, abdominal pain and diarrhea within two weeks. Inclusion criteria was to be pre-diagnosed as acute gastroenteritis. The patients who admitted to the hospital without gastrointestinal symptoms were excluded. Freshly taken stool samples sent to microbiology laboratory without any delay and primarily examined microscopically regarding the presence of blood-mucus and consistency of stool. The direct microscopic examination was done by serum physiologic and Lugol and the presence of parasite egg, trophozoites and cysts were explored. A commercial immunochromatographic rapid diagnostic test (sensitivity 84.8%, specificity 87.4%) based on antigen analysis in the stool (RIDA®QUICK Entamoeba, Germany) was used for the identification of Entamoeba histolytica.

The inoculation of the stool samples was done to Salmonella/Shigella plate (Premed, Turkey) for bacteriological examination, and suspected colonies were assessed after 24 hours of incubation. Also, a commercial rapid diagnostic test (Orient Gene Rota/Adeno Virus Rapid Test Cassette, UK) was used for virological investigation, and positivity for Rotavirus and Adenovirus was investigated (for rotavirus sensitivity 97.5%, specificity is 97.6%; for adenovirus sensitivity 80.7%, specificity 100%). The results of samples obtained between 17th August – 1st September and samples obtained two weeks before the outbreak were compared based on the number of examined and microorganism-detected stool.

Statistical analysis

All analysis were performed with the help of IBM SPSS (Statistical Package for Social Science) Statistics 21.0 software.The difference between groups from categorical variables was studied using Pearson Chi - Square test, and Fisher's exact chi - square test. In the study, p-value lower than 0,05 was considered as statistically significant.

RESULTS

From 440 patients who applied to emergency service with acute gastroenteritis diagnosis, 227 (51.5%) were female, and the youngest patient was two months old, and the oldest patient was 79 years old. The average age was 15.6 years old. According to this, children who are five years old or younger comprise 181 of patients (41.1%), and 159 of them (58.9%) is six years old or over. Rotavirus was detected in 15 of 16 patients who are under five years old (Table 1). The samples, which are inoculated to SS agar, were evaluated for the presence of the Salmonella and Shigella species. However, none of the samples showed these bacteria. Rotavirus was positive for 16 (3.6%) of all stool samples. We cannot detect adenovirus in any sample. We found parasite egg, cyst and/or trophozoites in 146 of the samples (33.2%) (Table 2). When parasitic and viral factors were assessed together, pathogenic microorganisms were detected in 178 of 440 examined stool sample (40.5%).

Table 1. Distribution of isolated microorganisms with respect to patient age

Age	Entamoeba histolytica	Blastocystis hominis	Giardia lamblia	Dientamoeba fragilis	Hymenolepis nana	Rotavirus
≤5 (n: 181)	41 (22.7%)	9 (5%)	15 (8.3%)	2 (1.1%)	2 (1.1%)	15 (8.3%)
6-10 (n = 60)	19 (31.7%)	9 (15%)	4 (6.7%)	1 (1.7%)	0	0
11-15 (n = 42)	5 (11.9%)	5 (11.9%)	2 (4.8%)	0	0	0
16-20 (n = 36)	13 (36.1%)	6 (16.7)	0	2 (5.6%)	0	0
21-25 (n = 35)	7 (20%)	8 (22.9%)	1 (2.9%)	0	0	0
26-30 (n = 14)	0	1 (7.1%)	0	0	0	0
30-79 (n= 71)	11 (15.5%)	9 (12.7)	1 (1.4%)	1 (1.4%)	0	1 (1.4%)
Total (n:440)	96 (21.8%)	44 (10%)	24 (5.4%)	6 (1.4)	2 (0.4)	16 (3.6%)

Table 2. Comparison of detected microorganism incidence during in outbreak period (September 1-14, 2015) with pre-outbreak period (August 17- September 1, 2015)

	August 17-September 1	September 1 to 14 (Epidemic Period)	p
Number of samples	275	440	
Pathogens			
Entamoeba histolytica	19 (6.9%)	96 (21.8%)	<0,001
Blastocystis hominis	12 (4.3%)	44 (10%)	0,006
Giardia lamblia	7 (2.5%)	24 (5.4%)	0,06
Rotavirus	3 (1%)	16 (3.6%)	<0,001
Adenovirus	N	N	
Salmonella spp	N	N	
Shigella spp.	N	N	

As a result of direct microscopic examination of stool samples from patients, we cannot observe erythrocytes and leukocytes. Table 3 shows the distribution of parasites with regard to serum physiologic test, Lugol test and test for antibody presence in stool. While Entamoeba histolytica, Blastocystis, Giardia lamblia, Dientamoeba fragilis were present in 96 (21.85%), 44 (10%), 24 (5.5%), 6 (1.4%) of the patients respectively, helminth egg (Hymenolepis nana) was observed only for two patient (0.4%). While 120 of the patients (27.3%) showed only single parasite presence, 22 of them (5.0%) showed two and four of them (0.9%) showed triple parasites simultaneously (Table 4)

Table 3. The distribution of the detected parasites (n= 440)

Microorganism	n (%)	The total number and percentage of Detection of a single microorganism
Entamoeba histolytica	72 (16.7)	96 (21.9)
Blastocystis hominis	28 (6.3)	44 (10)
Giardia lamblia	18 (4.1)	24 (5.5)
Dientamoeba fragilis	1 (0.2)	6 (1.4)
Hymenolepis nana	1 (0.2)	2 (0.4)
Giardia lamblia + Blastocystis hominis	1 (0.2)	
Hymenolepis nana + Giardia lamblia	1 (0.2)	
Blastocystis hominis + Entamoeba histolytica	15 (3.4)	
Giardia lamblia + Entamoeba histolytica	4 (0.9)	
Dientamoeba fragilis + Entamoeba histolytica	1 (0.2)	
Dientamoeba fragilis + Entamoeba histolytica + Blastocystis hominis	4 (0.9)	

Table 4. Single or simultaneous detection rate of parasites (n = 440)

	n (%)
Single parasite	120 (27.3)
Two parasites simultaneously	22 (5.0)
Three parasites simultaneously	4 (0.9)

DISCUSSION

AGE is one of the most common diseases throughout the world and is a major public health problem. Consumption of food or drinking water contaminated with feces is one of the leading causes of gastrointestinal disorders. Regarding to the reports from many countries, it is reported that mainly drinking water is the source of gastrointestinal outbreaks[8,9]. Many infectious agents including primarily Norovirus and Rotavirus may cause AGE either sporadically or epidemically[9]. In this study, application of 440 patients to emergency service with complaints of nausea, vomiting, abdominal pain and diarrhea within two weeks of September 2015 in Şırnak city was considered as outbreak and investigations were done to determine the causing agents. When the detected microorganism rates in outbreak period (September 1-14, 2015) was compared with the pre-outbreak period (August 17- September 1, 2015), a substantial increase can be seen regarding identified parasites and rotavirus incidence during the outbreak period.

Rotaviruses are the leading factor of gastroenteritis especially in children in worldwide. With the help of routine vaccination programs in recent years, hospitalization and death related with rotaviruses decrease significantly, especially in America and Europe. However, in developing countries, rotavirus infection maintains its importance[10]. In this study, within two weeks of outbreak 16 of the 440 patients in (3.6%) were found to be rotavirus positive. When results from the pre-outbreak period were examined, 3 of 275 patients (1%) were found to be rotavirus positive. Comparison of these results revealed that rotavirus positivity increased during the outbreak period.

The most common bacterial agents causing AGE can be listed as Campylobacter, Shigella, Salmonella, Escherichia coli, Bacillus cereus and Staphylococcus aureus[6]. On the other hand, Salmonella and Shigella existence cannot be determined for the outbreak in our city. This finding suggests that the experienced outbreak is not due to bacterial pathogens. We discovered structures such as parasite eggs, cysts and / or trophozoites in 146 (33.2%) of the samples. Parasite-related infections are more likely to be seen in developing countries due to relatively low socioeconomic status and poor hygienic conditions. 200 water-borne parasitic outbreaks have been reported between 2004-2010 worldwide. The parasites, which are mostly responsible for these outbreaks, are protozoans such as Cryptosporidium, Giardia, and Entamoeba species[11].

Cho et al. reported an outbreak of 124 cases followed by watery diarrhea and vomiting in 2012 in Korea in a multi story building. As a result of the microbiological investigation of water which is taken from building's water system, it is determined that the drinking water was contaminated with fecal bacteria and high incidence of Cryptosporidium was observed. Pollution of drinking water with sewage water was identified as the cause of the outbreak[12]. Since we have no opportunity to do staining for modified acid resistant pathogens, we did not investigate the presence of protozoans such as Cryptosporidium and Cyclospora in our study.

Giardia is one of the most common parasites that is leading to intestinal infections in humans. According to data of Center for Disease Control and Prevention (CDC), 242 outbreaks due to Giardia has been reported between 1971-2011 in the United States. While most of these outbreaks are water-borne, food-borne outbreak, outbreaks due to transmission from person to person and outbreaks after animal contact have also been reported[13]. In an outbreak, which is thought to be waterborne in Korea, G. lamblia was detected with a high incidence as 36 %[14]. In our study, we identified Giardia lamblia in 24 patients (5.4%), and it is the most commonly seen parasite after Entamoeba histolytica and Blastocystis. Entamoeba histolytica can be identified as the causative agent in gastroenteritis outbreaks as well[15].

In a report by Barwick et al. 177 patients was examined because of suspected amebiasis in Georgia in 1988 and 37 of 52 patients who had liver abscess were also positive for E. histolytica in their serum and 11 of them was identified for intestinal amebiasis. Drinking water was suspected as the source of infection[16]. Entamoeba histolytica was determined in 96 of our study samples and become the most common factor with 21.8% incidence.

Pathogenicity of Blastocystis hominis is still debatable today. Tuncay et al. examined 196 stool samples in an outbreak which is thought to be water-borne and they found G. lamblia, in 7 patients, B. hominis in 4 patients and H. nana in 1 patient[17]. However in our study occurrence of B. hominis was 4.3% before the outbreak while it reaches critical rates such as 10% during the outbreak. Therefore, B. hominis may be a possible pathogen in our study. B. hominis is thought to be responsible for waterborne outbreaks in many researches[15].

In our study, 146 stool samples which are determined positive for parasite 26 of them (17.8) was positive for two or more parasites. Düzyol et al. reported the presence of various parasites in 158 of 2337 cases (6.7%)[18]. In our study high incidence of coexistence of parasites can be associated with outbreak which is believed to be water-borne. Sewage water, which polluted the drinking / tap water, was thought to be able to infect the patients with more than one parasite. Even though the incidence of the presence of an individual parasite is high, the prevalence of coexistence of different parasites is also noteworthy[19]. Accordingly, it is crucial to take enough time through the parasitological examination of the samples. Distribution of the parasites determined in a neighboor city is; 6.6% G. lamblia, 3.2% plenty B. hominis, 1.3% Hymenolepis nana, 0.1% Entamoeba histolytica/Entamoeba dispar.[20] Because of the outbreak parasites rates of our city was higher.

Limitations of the study were not searching more microorganisms like Norovirus and Camphylobacter. Also trichrome stain or moleculer microbiologic methods could be used. Lack of PCR usage may indicate un(mis)differenciation between newly idenitified nonpathogenic Entamoeba species and Entemoeba histolytica. Because of an acute gastroenteritis and conditions of our laboratory we could not perform these tests.

Consequently, we thought that the reason for this outbreak occurred in Şırnak city is pollution of drinking water with sewage water and lack of adequate water purification. During outbreak people were warned not to drink tap water and waters were super chlorized in order to end the outbreak by municipality.In order to prevent such diseases human and animal wastes must not contact with drinking water and food and the sterilization of the drinking water appropriately is very crucial.

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