Prevalence of Microorganisms in Patients Presented with Gastroenteritis to the Emergency Department

Acil servise başvuran gastroenteritli hastalarda etken mikroorganizmaların prevalansı

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SUMMARY

Objectives: Gastroenteritis is an inflammatory disorder of gastrointestinal mucosal surface that begins with symptoms and/or sings such as anorexia, nausea and vomiting and manifested with diarrhea of varying degrees and discomfort of abdomen. Acute gastroenteritis is the most affecting diseases in all ages. Determining the probable agents of gastroenteritis provides an easy diagnosis and effective treatment with appropriate antimicrobial agent. The aim of this study was to determine the pathogens in patients presenting with diarrhea to the emergency department.

Materials and Methods: In order to determine the etiologic agents of acute gastroenteritis, patients presented with diarrhea to Sivas Cumhuriyet University Medicine Faculty Emergency Department between May and November 2005 were included in the study. The stool analysis of the study patients was performed to detect bacteria, parasites, viruses and fungi.

Results: The study group consisted of 79 (52.7%) males and 71 (47.3%) females. In 61 (40.6%) patients the microorganism species were determined. Defined microorganisms were as follows; *Giardia intestinalis* 12 (8%), *Blastocystis hominis* 10 (6.7%), *Salmonella* 10 (6.7%), *Entamoeba histolytica* 9 (6%), *Rotavirus* 7 (4.7%), *Shigella* 5 (3.3%), *Clostiridium difficile* 2 (1.3%), Adenovirus 2 (1.3%), *Cryptosporidium* 2 (1.3%) and *Candida* 2 (1.3%). Fatigue (55 patients) was the most seen symptom in the patients whose pathogen microorganism was identified. Nausea-vomiting (50 patients) and abdominal pain (49 patients) followed it. Fever was the least seen symptom which was only seen in 32 (5%). Antibiogram analyses were performed for *Salmonella* and *Schigella* species. All of them were resistant to amikacin and tobramicine. Aztreonam was effective in all. Ciprofloxacin was effective in 14 patients.

Conclusion: The prevalence of protozoa was found to be significantly higher than the rotaviruses and bacteria among patients with gastroenteritis in this region.

Key words: Diarrhea; emergency department; gastroenteritis; pathogen.

ÖZET

Giriş: *Gastroenterit, iştahsızlık, bulantı ve kusma gibi semptomlarla başlayan, değişik derecelerde ishal ile kendini gösteren ve abdominal rahatsızlık hissinin eşlik ettiği gastrointestinal mukozanın enflamatuvar bir hastalığıdır. Akut gastroenteritler bütün dünyada her yaştan insanda görülebilen ve kişilerin günlük yaşamını en çok etkileyen hastalık grubudur. Etken mikroorganizmanın belirlenmesi uygun antimikrobiyal ajan ile erken ve etkili tedavi sağlar. Bu çalışmanın amacı, acil servise başvuran ishalli hastalarda etken patojenleri belirlemektir.*

Gereç ve Yöntem: *Çalışma Sivas Cumhuriyet Üniversitesi Tıp Fakültesi Acil Servisi'ne başvuran akut gastroenteritli 150 hasta ile Mayıs-Kasım 2005 tarihlerinde yapıldı. Etyolojik ajanların belirlenmesi amacıyla çalışma hastalarının dışkı örneklerinde bakteri, parazit, virüs ve mantar incelemeleri yapıldı.*

Bulgular: *Çalışmaya 79 (%52,7) erkek, 71 (%47,3) kadın hasta dahil edildi. Bunların 61'inde (%40,6) patojen etken belirlenirken, 89'unda (%59,4) ise belirlenemedi. İncelemeler sırasında 150 dışkının 12'sinde Giardia intestinalis (%8), 10'unda Blastocystis hominis (%6,7), 10'unda Salmonella (%6,7), 9'unda (%6) Entamoeba histolytica, 7'sinde (%4,7) Rotavirüs, 5'inde (%3,3) Shigella, 2'sinde Clostiridium difficile (%1,3) 2'sinde* adenovirüs *(%1,3) 2'sinde (%1,3) Cryptosporidium ve 2'sinde de (%1,3) Candida türü saptandı. Etken patojenin saptanabildiği hastalarda en sık semptom yorgunluktu (55 hasta). Bulantı-kusma (50 hasta) and karın ağrısı (49 hasta) yorgunluğu izledi. Ateş en az görülen semptomdu (32 hasta). Antibiyogram sadece Salmonella ve Shigella türleri için yapıldı. Salmonella ve Shigellanın etken olduğu tüm hastalar amikazin ve tobramisine duyarlı dirençli*

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olmalarına rağmen, aztreonam bütün hastlalarda etkiliydi. Siprofloksazin ise 14 hastada etkiliydi.

Sonuç: *Bölgemizde protozoonların prevalansı bakteri ve rotavirüse göre anlamlı oranda daha yüksektir.*

Anahtar sözcükler: *Acil servis; gastroenterit, ishal; patojen.*

Introduction

Gastrointestinal infections reveal generally as gastroenteritis. The major symptom is diarrhea. Gastroenteritis (GE) is seen throughout the world. The prevalence of GE varies according to the climate, environmental conditions, sewer system affectivity, and the level of the public's education of health. Furthermore, it is an important cause of morbidity and mortality in the developing countries. Bacteria and parasites are the common agents that are detectable; however viruses are usually the undetected agents.[1]

The etiological agents for GE differ according to the geographic regions. Technological developments enable us to detect different pathogens in GE. Pathogens generally contaminate with water or food sources, that's why a lot of people can be infected with the same pathogen.[2]

A lot of improvement is accessed about the virulence, physiopathology and treatment of the infectious diarrhea. However, it is getting more difficult to treat infectious diarrhea due to the drug resistance. Usually GE improves only with supportive treatment. Nevertheless, empirical antibiotic treatment in acute gastroenteritis is a common habit. Unnecessary use of antibiotics increases costs, antibiotic resistance and may cause adverse effects which indicates the importance of detecting the microorganisms in GE to avoid these problems.

In Turkey, a big part of patients with GE patients admit to the emergency departments (ED). Identification of the microorganism species in our area helps us in rapid diagnosis and early treatment. The aim of this study was to determine the pathogens in GE in order to establish cost-effective treatment modalities.

Materials and Methods

This prospective study was performed in the ED of Cumhuriyet University Hospital between May and November 2005. The local ethics committee approved the study.

Patients, between 16-85 years presented with acute GE to ED the ED were included in the study. Stool species taken from the study patients were examined for bacteriologic, parasitic and viral agents. Patients with complaints more than 14 days were accepted as chronic GE and excluded from the study.

In order to detect the bacterial agents;

a. Stool examination with gram staining: All of the fresh stool species were gram stained and examined in the microbiology unit.

b. Culture: Stool species were inoculated in EMB, blood agar, SS and selenit-F plates. After 18-24 hour incubation in 37 °C the detected lactose (-) colonies were inoculated in TSI. Then they were taken for identification process. Culture, identification and antibiogram processes were made in beckton-dickson plates with phoenix model instrument. At least 48 enzymatic parameters were used for identification. Antibiograms were performed according to minimum inhibitor concentration (MIC).

Because of the *Clostridium* species reproduce in anaerob bloody agar and identification was performed by beckton dickson.

c. Toxin A test: Veda-LAB (France) kits were used for *Clostridium difficile* toxin A. The process was performed manually and results were taken in 10 minutes.

For parasitologic examinations a smear of feces diluted with 0.9% saline was examined microscopically with 10X and 40X magnifications. Iodied was added to specimens in order to isolate the protozoon cysts. For detection of *Cryptosporidium* oocysts, smears were prepared with Kinyoun acid fast staining method.[3-5]

All stool species were evaluated for adeno and rotaviruses with adeno-heck-1 and rota-heck-1 instruments from France Veda lab according to the manufacturer instructions and the results were taken in 10-15 minutes.

After the inoculation of stool species in the mediums, specimens in which only candida species grew were taken for fungal evaluation.

The determined agent rates were classified according to the sex and age groups. All of the patients were classified according to their ages. Each group was composed of a 10-year-age interval and the first group included 16-25 years. Also the rate of microorganism types, cultural results, the relation between the clinical symptoms and pathogen microorganisms were evaluated. Ultimately, antibiogram affectivity for the pathogens was determined.

Table 1. *Distribution of the patients according to their age groups.*

Age	16-25	26-35	36-45	46-55	56-65	66-75	75-85
Number	38	28	29	20	16	14	5
%	25.4	18.7	9.3	13.4	10.7	9.3	3.3

Table 2. *Identification rate of patients with gastroenteritis.*

Variables	Male		Female		Total	
	Number	%	Number	%	Number	%
Identified pathogen	36	24	25	16.6	61	40.6
Nonidentified pathogen	43	28.7	46	30.7	89	59.4
Total	79	52.7	71	47.3	150	100

The study data were analyzed with SPSS 12.0 for Windows. The variables were expressed as rates and the comparison of categorical data was performed by Chi-Square test. All the hypotheses were constructed two tailed and the critical alpha value was accepted as 0.05.

Results

Stool species from 150 adult patients who admitted because of acute GE to the ED were examined. Gastroenteritis were mostly seen in group 1 (16-25 age, 25.3%), group 3 (36-45 age, 19.3%) and group 2 (26-35 age, 18.7%). The lowest rate of GE was in group 7 (75-85 age, 3.3%). Table 1 displays the distribution of the patients according to their age groups 52.7% (n=79) of the study patients were men and 47.3% (n=71) of them were women. The pathogen could be identified only in 61 patients (40.7%). The pathogens were identified in 24% (n=36) of males and 16.6% (n=25) of females (Table 2). The prevalence of identified pathogens was not statistically different according to the gender ($p>0.05$).

Protozoons were the most detected microorganisms. Then bacteria, viruses and fungi followed-up protozoon. The mostly established protozoon was *Giardia intestinalis* 8% (n=12), *Blastocystis hominis-Salmonella* species 6.7% (n=10) and *Entamoeba histolytica* 6% (n=9). The least seen protozoon was *Cryptosporidium* 1.3% (n=2). Adenoviruses and *Clostridium difficile* were seen as in the same rate with *Cryptosporidium*. Table 3 shows the rate of identified microorganisms and Table 4 shows the rate of microorganism groups.

Only 11.3% of stool cultures were positive for bacterial and fungal species. In 2 patients *Candida* species were grown up alone. *Schigella boydii* and *Schigella sonnei* species were the types that were grown up in aerop culture mediums. Table 5 shows the culture results.

Table 3. *The prevalence of identified microorganisms.*

Pathogen	Number	%
Salmonella species	10	6.7
Shigella species	5	3.3
C. difficile	2	1.3
Rotavirus	7	4.7
Adenovirus	2	1.3
E. histolytica	9	6
G. intestinalis	12	8
B. hominis	10	6.7
Criptosporidium	2	1.3
Candida species	2	1.3
Non identified	89	59.4
Total	150	100

Table 4. *The rate of microorganism groups.*

Pathogen group	Number	%
Protozoa	33	54.1
Bacteria	17	27.9
Virus	9	14.7
Fungi	2	3.3
Total	61	100

Table 5. *Culture results.*

Culture result	Number	%
Normal flora	133	88.7
Salmonella spp.	10	6.7
Shigella spp.	5	3.3
Pure *Candida*	2	1.3
Total	150	100

Table 6. *Corelation between the clinical symptoms and identified microorganisms.*

Pathogens	Fever	Abdominal pain	Nausea-vomiting	Fatigue	Tenesm
Salmonella spp.	6	9	9	10	5
Shigella spp.	4	4	5	4	4
C. difficile	2	2	2	2	2
Rotavirus	6	2	3	7	1
Adenovirus	1	2	2	2	1
E. histolytica	3	7	8	8	6
G. intestinalis	3	12	9	12	9
B. hominis	5	5	6	8	3
Criptosporidium	1	2	2	2	1
Candida spp.	0	2	2	2	0
Total	32	49	50	55	33

Fatigue was the most seen symptom in the patients whose pathogen microorganism was identified. Nausea-vomiting and abdominal pain followed it. Fever was the least seen symptom which was only seen in 32 patients. Table 6 shows the corelation between the clinical symptoms and identified microorganisms.

Antibiogram analyses were performed for *Salmonella* and *Schigella* species. All of them were resistant to amikacin and tobramicin. Aztreonam was effective in all. Ciprofloxacin was effective in 14 patients. Table 7 shows the antibiogram results.

Table 7. *Antibiogram results.*

Antimicrobial agent	Sensitive	Mild sensitive	Resistant
Amikacin	0	0	15
Amoxicillin/ Clavulanate	0	5	10
Ampicillin	7	0	8
Aztreonam	15	0	0
Cefepime	13	1	1
Cefotaxime	12	1	2
Cefoxitin	0	2	13
Ceftazidime	12	2	1
Cefuroxime sodium	0	2	13
Cephalotin	0	2	13
Ciprofloxacin	14	1	0
Gentamicin	0	2	13
Levofloxacin	14	1	0
Meropenem	13	2	0
Piperacillin	10	3	2
Piperacillin/ Tazobactam	11	3	1
Tetracycline	1	1	6
Tobramycin	0	0	15
TMX/SMX	10	0	5

Discussion

Gastroenteritis is the second leading cause of mortality after cardiovascular disease in all ages. In the non-developed countries gastroenteritis are the major causes of death. Every year 4.600.000-6.000.000 children die from gastroenteritis in Asia, Africa and Latin America. That means that 10.000 children die in a day or seven children in every minute. Most of the mortalities occur in developing or non-developed countries because of pauperism, insufficient fresh water and starvation.[6,7]

The frequency, severity and type of gastroenteritis depend on the patients immun system, life conditions and seasons. The prevalence of gastroenteritis also depends on the age, life style and cultural habits. Adults can be asymptomatic carrier of microorganisms. The residence type, warren, deficiencies in sanitization and insufficient fresh water are the major sources of pathogens which causes gastroenteritis. Geographical regions and climates determine the pathogens and disease characteristics.[3,4,6,7]

Rotaviruse is the major cause of viral gastroenteritis. Yıldırmak et al.[8] found the prevalence of rotaviruse as 29% in 106 nurslings. Türkoğlu et al.[9] found the prevalence as 25.4% by using enzyme linked immunosorbent assay (ELİSA) latex agglutination in 826 children with gastroenteritis. Caşkurlu et al.[10] didn't find any rota or adenoviruses by using latex agglutination in 100 patients with gastroenteritis. Başustaoğlu et al.[11] found the rotavirus rate higher in winter. The rotavirus prevalence had been reported higher in children by Seo JK and Sim JG in Korea between September and February.[12]

Kocazeybek et al.[13] examined 1900 stool species between January 1999 and June 2000. Six hundred fifty one patients

were investigated for rotaviruse and 143 (22%) species were found positive by using latex agglutination test. In our results, this rate was 4.7%. The difference may be explained by the differences in the study periods and the ages of the study populations that the present study evaluated patients between 16 and 82 years. In developed countries, the prevalence of bacterial pathogens is between 4-14%. However, in our country this changes according to the geographical region and socioeconomic level of the population. In 1994, a research was conducted in Göztepe Education Hospital and the prevalence of bacterial pathogens in gastroenteritis was found as 20.4% among 1215 patients.[14] Another study was made in 1999 in Ankara by Refik Saydam Hıfzısıhha research laboratory and the bacterial prevalence was 13.7%.[14] The prevalence of bacterial pathogens in our study was 11.3%.

Yurdakök et al. examined 19.812 stool species and found 618 *Shigella* species (3.2%).[15] They also examined stool species of 20.100 children with gastroenteritis between 1987 and 1994 and found 508 (2.5%) *Salmonella* species.[16] Aktaş et al.[17] found *Campylobacter jejuni* as the major pathogen (8.8%) in 125 adult patients. The rate in the study by Işık et al. for *Campylobacter jejuni* was 7.5% (n=6).[18] Kanan et al.[19] examined 317 species of patients with gastroenteritis and found only two species of *Campylobacter*. In the same study the prevalence of *Shigella* and *Salmonella* were 0.81% (n=3) and 3.26% (n=12).[19] Özgüneş et al.[20] determined the rate of *Salmonella* and *Shigella* species as 10.9% and 8.8% among the patients with gastroenteritis in İzmir.

Akçam et al.[21] examined the stools of 81 patients and one patient had *Salmonella paratyphi* A and one patient had *Shigella sonnei*. The bacterial pathogen species rates in the study by Karaer et al. were as follows: *Escherichia coli* 7.2%, *C. jejuni* 6.1%, *C. difficile* 5.2%, *Salmonella* species 3.5% and *Shigella* species 2.4%.[22] In the present study, the prevalence of the bacterial pathogens was as follows: *Salmonella* 6%, *Shigella* 2.7%, *E. coli* 1.3% and *C. difficile* 1.3%. Two of the patients among the four *Shigella* species had *Shigella sonnei* and two had *Shigella boydii*. *Shigella sonnei* is known as the pathogen of industrial countries pathogen, whereas *Shigella flexneri*, *Shigella dysanteria* and *Shigella boydii* are mostly found in developing countries. Nevertheless, the prevalence rates change according to the immunization rate to *Shigella* species. In the present study, we couldn't find any *S. flexneri* type.

A high antimicrobial resistance was evident in the present study. Ampicillin and trimetoprim-sulphametaksazol are used in a wide range for empirical treatment in gastroenteritis. But the high antimicrobial resistance has reduced their successful usage.

The antimicrobial results showed us that quinolons and 3rd class cephalosporins are beneficial in bacterial gastroenteritis. In our department ciprofloxacin is used for bacterial pathogens for empirical treatment. As a result, antibiogram is necessary for a real treatment in bacterial gastroenteritis.

Taş et al.[23] investigated the thermophylic *Campylobacter* species, *E. coli* and rotavirus prevalence in order to determine if it is necessary for routine examination. According to their results, they suggested that a routine examination is necessary for *Campylobacter* species in patients with gastroenteritis whose direct examination included leucocytes and occur in summer. They found also that rotaviruse examination is necessary in children and geriatric patients with gastroenteritis in winter.

The prevalence of *Campylobacter* species had been found similar to the *Salmonella* and *Shigella* species in the prior mentioned study. The prevalence was higher among children and in summer. In our examinations we didn't find any *Campylobacter* species. This may be due to the investigation of only adult patients. *Yersinia enterocolitica* is another cause of bacterial gastroenteritis and is usually found in cold regions. In our study we didn't find any *Yersinia* pathogens. This can be explained due to the study period which was between May and October.

Cryptosporidosis is common throughout the world but is more in developing countries. The healthy people improve by themselves but in patients with AIDS, chronic renal failure who need dialysis or with the other disease that compromise the immun system *Cryptosporidosis* can be mortal. In the patients with *Cryptosporidosis*, abdominal pain, diarrhea, nausea and vomiting are the usual symptoms. Fever muscle pain, fatique, headache and lack of appetite can also be seen.[24-27]

The *Cryptosporidium* rate in our country changes according to the regions. Akyon et al. found the prevalence as 3% in children with gastroenteritis whereas in healthy group it was 0%.[28] The prevalence in neoplastic patients is determined by Tanyüksel et al. as 17%; however they did not find any *Cryptosporidium* oocystes in neoplastic patients.[29] The *Cryptosporidium* prevalence varies.[30] Yücel et al.

reported a prevalence of 1.03%,[30] Atambay et al. 1.6%,[31] İnceboz et al. 0.4%,[32] and Özçelik et al. 11.8%.[33] In a study in Korea, nine *Cryptosporidium parvum* oocysts had been detected in 461 patients with gastroenteritis.[34] Sarı et al.[35] examined the stool species of patients with chronic renal failure and found 3 oocysts among 47 patients by using Kinyoun acid fast method. In the present study, the *Cryptosporidium* rate was 1.3% (2 patients). Diarrhea due to the *Cryptosporidium* has been seen throughout the world and the prevalence varies according to the regions. It is also seen in all age and sex groups. That's why parasitic examination is necessary for gastroenteritis in order to treat the patients well.

Jelinek et al.[36] investigated 469 patients with gastroenteritis for *B. hominis* and *Entamoeba histolytica*; the prevalence rates were 14.7% and 8.7%, respectively. Doğan et al.[37] examined 537 stool species in Eskişehir and determined 88 patients with *B. hominis*. The rates differ in different studies. Üner et al.[38] reported the rates of *G. intestinalis*, *E. histolytica* and *B. hominis* as follows: 12.3%, 1.6% and 37%, respectively. Kocazeybek et al.[13] determine the prevalence of *E. histolytica* as 7.8%. In a study carried out by Eren et al.[39] in Sivas, the rates were 8% for *G. intestinalis*, 6% for *E. histolytica* and 2% for *B. hominis*. Üstün et al.[40] reported the rate of *E. histolytica* as 0.98% and 0.25% for *B. hominis*.

In a study between 1999-2001 which was carried out in the Parasitology Department of İnönü University, 500 stool species were examined and the protozoon rates were 6.2% for *G. intestinalis*, 2.8% for *E. histolytica* and 2.2% *B. hominis*.[41] Stool species were examined with direct microscopy (X400) which five or more *B. hominis* were accepted as positive.[41] Üner et al.[42,43] analyzed 100 stool species and *E. histolytica* rate was 1%. In another study with 248 stools examined and the prevalence rates were 12.9% for *G. intestinalis* (32 patients), 1.6% (4 patients) for *E. histolytica* and 37% for (92 patients) *B. hominis*. The high rate for *B. hominis* was concluded due to the non-hygienic life conditions. In the present study, the rates were 8% (12 patients) for *G. intestinalis*, 6.7% (10 patients) for *B. hominis* and 6% (9 patients) for *E. histolytica* and *Salmonella* species.

Caşkurlu et al.[10] investigated 100 patients with gastroenteritis according to their clinical symptoms, etiologies and treatments who were hospitalized in the Infection Disease Department. The symptoms rates were as follows: 42% fever, 57% abdominal pain, 20% tenesm and 65% nausea and vomiting. The fever rate was 21% in another study by Jelinek et al.[36] Demirdağ et al.[44] reported the symptoms in intestinal amebiasis as 72.5% nausea, 52.5% tenesm, 47.5% vomiting and 32.5% fever. In the present study the most common symptom was fatigue. Nausea, vomiting and abdominal pain were the other symptoms that were mostly seen. Fever, only seen in 32 (21.3%) patients, was the least frequent symptom.

Gastroenteritis due to the *Candia* occurs especially after antibiotic use. Kocazeybek et al.[15] and Karaer et al.[24] reported the rates of *Candida albicans* as 2.4% and 7.6%. In the present study, we have accepted the patients with pure *Candida* species in their stool species as fungal gastroenteritis. The rate was 1.3% and no one had prior usage of an antibiotic or an immuncompromise state.

As a result of this study, we determined the protozoon rate as important as bacteria and viruses rates in gastroenteritis. The drug resistance rate was also high in the present study; it may be a result of the common empirical treatment habit. The epidemiological data about the prevalence of microorganism causing gastroenteritis in your region and their seasonal distribution should be important in avoiding the unnecessary antibiotic usage, drug resistance and in decreasing costs.

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