KAFKAS TIP BİLİMLERİ DERGİSİ
Kafkas Journal of Medical Sciences

ARAŞTIRMA YAZISI / ORIGINAL ARTICLE

Kafkas J Med Sci 2016; 6(2):94–97 • doi: 10.5505/kjms.2016.30301

Acute Gastroenteritis Agents Among 0–5 Years-Old Turkish Children

0–5 Yaş Arası Türk Çocuklarda Akut Gastroenterit Etkenleri

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ABSTRACT

AIM: *Acute gastroenteritis outbreaks are the common health problem throughout the world especially in children. Every year, thousands of children die due to the diarrhea caused by bacteria, parasites and viral agents. In this study, we aimed to evaluate the rates of diarrheal agents in 0–5 years-old children's stool samples in terms of seasons.*

METHODS: *In this study, 216 stool samples were taken from 0–5 year-old children. These samples were examined with some tests for Rotaviruses, Adenoviruses, Salmonella, Shigella, Entamoeba, Giardia, Clostridium difficile Toxin A and Toxin B. Clostridium difficile toxins were detected by using ELISA (CerTest, Biotec, Spain). Immunochromatographic tests were used to detect the Rotavirus, Adenovirus, Giardia and Entamoeba antigens. Selenite-F broth and MacConkey Agar mediums were used to find lactose negative colonies for Salmonella and Shigella in stool samples. Confirmation was performed by IMVIC tests.*

RESULTS: *The obtained results showed that the isolated agents were viral (40.74%), bacterial (24.98%) and parasitic (20.82%) respectively. Some agents showed a peak in the cold seasons such as Rotavirus (November, December, January and February). On the other hand, some outbreaks that came out by Salmonella and Shigella were seen most frequent in hot seasons (June, July, and August).*

CONCLUSION: *According to the obtained data, diarrheal infections were mostly identified in February, March and April. Rotavirus infections are more frequent in winter and spring. In conclusion; we believe that analysis of viral antigens, bacteria and the parasites as diarrheal agents in stool sample is important in 0–5 years-old infants to prevent hospitalizations and unnecessary drug use.*

Key words: *children; diarrhea; gastroenteritis; rotavirus; salmonella; giardia*

ÖZET

AMAÇ: *Akut gastroenterit salgınları bütün dünyada özellikle çocuklar için ortak bir sağlık problemidir. Her yıl binlerce çocuk bakteri, parazit ve viral etkenlerin sebep olduğu diyare sonucu hayatını kaybetmektedir. Bu çalışmada, 5 yaş altı çocukların dışkı örneklerinde mevsimlere göre diyare etkenlerinin oranlarının bulunması hedeflenmiştir.*

YÖNTEM: *Bu çalışmada, 0–5 yaş arası çocuklardan 216 gaita örneği toplanmıştır. Bu örnekler; Rotavirus, Adenovirus, Salmonella, Shigella, Entamoeba, Giardia, Clostridium difficile Toksin A ve Toksin B testlerine tabi tutulmuştur. Clostridium difficile toksinleri ELISA metodu ile tespit edilmiştir. Rotavirus, Adenovirus, Giardia and Entamoeba ajanlarına ait antijenlerin tespiti için İmmunkromatografik testler kullanılmıştır. Dışkı örneklerinde Salmonella ve Shigella şüpheli laktoz negatif kolonilerin tespiti için MacConkey Agar ve Selenit-F buyyon kullanılmıştır. Doğrulama için IMVIC testleri yapılmıştır.*

BULGULAR: *Elde edilen sonuçlar, izole edilen ajanların sırasıyla viral (%40,74), bakteriyel (%24,98) ve parazitik (%20,82) ajanlar olduğunu göstermiştir. Rotavirus gibi bazı ajanlar en çok kış aylarında (Kasım, Aralık, Ocak ve Şubat) en yüksek seviyede tespit edilmiştir. Diğer taraftan, Salmonella ve Shigella gibi bazı bakterilerin sebep olduğu salgınlar ise yaz aylarında (Haziran, Temmuz ve Ağustos) daha sık görülmüştür.*

SONUÇ: *Elde edilen verilere göre, diyare enfeksiyonları genellikle Şubat, Mart ve Nisan aylarında görülmektedir. Kış ve ilkbahar aylarında en sık görülen ajan Rotavirus'tur. Sonuç olarak, hastaneye yatış ve gereksiz ilaç kullanımının önüne geçilebilmesi için 0–5 yaş arası çocukların dışkı örneklerinde viral, bakteriyel ve parazitik ajanların diyare etkeni olarak araştırılmasının önemli olduğuna inanmaktayız.*

Anahtar kelimeler: *çocuk; diyare; gastroenterit; rotavirus; salmonella; giardia*

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Geliş Tarihi: 15.02.2015 • Kabul Tarihi: 27.07.2015

Introduction

Acute gastroenteritis is one of the most common health problems all over the world[1]. Acute diarrheal infections are also a common disease in children. Estimated incidence rates in developing countries are between 3.5 and 7.0 episodes per child per year during the first 2 years of their life while they are between 2 and 5 episodes per child per year for the first 5 years[2]. Pediatric diarrhea is an important disease and it may lead an emotional trauma for the child and their parents[3].

More than 700 million cases are annually estimated to occur in 0-5 year old children, resulting in few deaths in developed countries. On the other hand, this rate is about 2 million deaths in developing countries. A group of viral, bacterial, and parasitic pathogens cause acute enteric symptoms including nausea, vomiting, abdominal pain, fever, and acute diarrhea. Until the early of 1970s, most viral agents causing gastroenteritis in humans were largely unknown. However, studies using electron microscopy of intestinal contents has been resulted in the discovery of numerous viral enteropathogens such as Rotaviruses, 'enteric' Adenoviruses or other viruses which may cause gastroenteritis[1]. Among them, viral infection is the most common cause, followed by bacterial and parasitic infections[4]. *Giardia lamblia* and *Entamoeba histolytica* are the major parasitic agents for diarrhea[5].

Salmonella spp. and *Shigella spp*. are the bacterial agents which are mostly isolated from stool samples of diarrheal patients, especially in rural areas from developing countries[6,7]. Additionally, *Clostridium difficile*, another bacterial diarrheal agent, is a big threat for children in both community and hospitals[8,9]. *Clostridium difficile* infection has more recently been implicated as dramatically increased prevalent diarrheal pathogen in children[10-12]. Moreover, evidence suggests that a large proportion of pediatric *Clostridium difficile* cases are community-acquired infections[13,14]. In this study, our aim was to detect the prevalence of these gastroenteritis agents in 0–5 year-old children.

Materials and Methods

216 diarrhea-diagnosed children who were admitted to consecutively to the hospital were included to this study. Children who had been treated with antibiotics before the onset of diarrhea were also included. Samples were collected into a sterile sample cup and were transported the same day to hospital laboratories, where they were stored at 4–8°C until they were processed. Specimens for bacteriological culture were inoculated into appropriate media immediately.

Stool specimens from each child with severe gastroenteritis were tested for *Clostridium difficile* by ELISA method to detect Toxin A an Toxin B. *Rotavirus* and *Adenovirus* Card Tests (CerTest, Biotec, Spain), a qualitative immunochromatographic assay were used to detect *Rotavirus* and *Adenovirus* antigens. This immunochromatographic tests were also used for detecting *Giardia* and *Entamoeba* antigens. The stool samples were examined under the microscope after the card test in terms of *Giardia* and *Entamoeba*. Selenite-F broth and MacConkey Agar were used to detect lactose negative colonies for *Salmonella* and *Shigella*. Finally, we performed IMVIC tests to confirm the possible positive samples. "I" is for indole test; "M" is for methyl red test; "V" is for Voges-Proskauer test, and "C" is for citrate test. These tests were performed in the Microbiology Laboratory by using appropriate mediums.

Results

The total number of children was 216 who applied to the hospital in 1 year with the complaints of gastroenteritis. *Rotaviruses* were the most isolated pathogen. According to the seasonal data, the peak incidence occurred in February (n=27) and August (n=28). Additionally, Adenoviruses were detected in 29 of the total samples (13.42%) and *Clostridium difficile* strains isolated from 30 of 216 (13.88%). *Giardia* and *Entamoeba* were identified in 19 (8.79%) and 26 (12.03%) respectively (Table 1).

Some agents are mostly isolated during winter months (December 14.77%, January 13.76%, February 25%) such as *Rotavirus*. Five Adenovirus-Rotavirus mix infections were detected (2 cases in February, 1 case in March, and 3 cases in April). On the other hand, some outbreaks that came out by Salmonella (June 21.42%, July 28.57%, August 35.71%) and Shigella (June 20%, July 20%, August 30%) were seen most frequently in hot seasons (Table 1).

Discussion

Acute gastroenteritis in children continues to be a significant health problem throughout the world. Millions of cases of acute diarrheal disease are estimated to occur annually just in 0–5 year old children[15].

Table 1. *The distribution of isolated diarrheal agents*

	January	February	March	April	May	June	July	August	September	October	November	December	**TOTAL**
Agents	n	n	n	n	n	n	n	n	n	n	n	n	**n**
Giardia spp.	1	0	2	2	1	3	3	4	0	1	2	0	**19**
Entamoeba spp.	0	1	1	3	2	4	5	6	2	0	1	1	**26**
C.difficile	2	2	0	3	2	3	4	7	6	1	3	0	**30**
Salmonella spp.	0	0	0	0	1	3	4	5	1	0	0	0	**14**
Shigella spp.	1	0	0	0	1	2	2	3	0	1	0	0	**10**
Adenovirus	0	2	2	3	2	4	2	2	3	3	2	4	**29**
Rotavirus	12	22	8	4	3	0	0	1	7	8	10	13	**88**
Total number	**16**	**27**	**13**	**15**	**12**	**19**	**20**	**28**	**16**	**14**	**18**	**18**	**216**

African children accounted for the biggest part (42%) of total 10.6 million deaths among 0–5 years-old children in the world[16].

Some studies state that enterotoxigenic *Rotaviruses* predominates in developing areas, cytotoxigenic *Clostridium difficile* are detected with increasing frequency in developed areas; and *Shigella, Salmonella* and *Giardia lamblia* are found whole the world[17]. In Netherlands, viral agents (especially *Rotavirus*) were isolated in the rate of 82% from stool samples while bacterial and parasitic agents were isolated in the rates of 32% and 10% respectively[18]. Our study is consistent with current literature. Rotaviruses were isolated in 40.74% (*Adenovirus* 13.42%, 5 cases are mixed infections) while these rates were 24.98% and 20.82% for bacteria and parasites. Most of the studies have mentioned that mixed infections were less frequent than mono-infections. A study that performed in Spain showed that the most frequent mixed infections were *Rotavirus-Astrovirus* (13 cases) and *Rotavirus-Adenovirus* (10 cases in 820 stool samples) infections[19].

This study found that the highest proportion of dual infections was identified in February, March and April months and seasonal occurrence of some mono-infections such as Rotavirus is more frequent in winter and spring. At the same study that performed in Spain, most of the cases with mixed infection occurred in autumn (26 cases in autumn, 5 in winter, 6 in spring, 2 in summer), and no seasonal differences were detected between the different co-infections[19]. As seen in the present study, *Rotavirus* is the most common isolated viral agent (40.74%) for acute childhood diarrhea. These findings are nearly same with another study performed in İzmir (*Rotavirus,* 39.8% in 920 children)[20]. On the other hand, *Clostridium difficile* infections (CDI) rate were detected as 13.88% in our study. Some researchers have found the incidence of CDI in the pediatric population increased in US hospitals[21].

Entamoeba histolytica, *Giardia lamblia* and *Cryptosporidium parvum* are considered to be the most important diarrheal agents[22-26]. We tried to identify *Entamoeba* and *Giardia* infections in our study. *Giardia lamblia* infections are very common throughout the world and are considered one of the main non-viral causes of diarrhea in industrialized countries[26]. For many years, microscopic examination of stool samples has been considered as "gold standard" for diagnosis of *Entamoeba histolytica*, *Giardia lamblia* and some parasites. Recently, more specific and sensitive alternative methods (PCR, ELISA) have been introduced for all these parasitic infections. We have detected these parasites with microscopic examination and according to the obtained data, *Giardia spp.* and *Entamoeba spp*. were detected in the rate of 8.79% and 12.03% of all samples respectively. These rates show us that parasitic infections are incontrovertible cause of acute diarrhea.

As a conclusion, we believe that analysis of viral antigens, bacteria and the parasites as diarrheal agents in stool sample is important in 0–5 years-old infants to prevent hospitalizations and unnecessary drug use.

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