Detection of Rotavirus and Enteric Adenovirus Antigens in Outpatients with Gastroenteritis

Ayaktan İzlenen Gastroenteritli Hastalarda Rotavirüs ve Enterik Adenovirüs Antijenlerinin Araştırılması

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ABSTRACT Objective: One of the most important reasons of mortality, especially in childhood, is infectious diarrheas. As their epidemiology is not well known in Turkey, the aim of this study is to characterize the distribution of gastroenteritis caused by rotavirus and adenovirus by age groups. **Material and Methods:** Determination of rotavirus and adenovirus antigens in stool samples of 2.962 patients admitted to MESA hospital with the diagnosis of acute gastroenteritis between January 2005 and June 2008 as carried out by the immunochromatographic method. **Results:** 605 of 2.962 stool samples were found to have viral antigens. 483 of those positive samples were found to be positive for rotavirus, and 77 of them were found to be positive for adenovirus. Forty five of the samples tested were positive for both virus antigens. Additionally, rotavirus and adenovirus associated gastroenteritis was observed most frequently in the 0-5 year-old group; and rotavirus was more common during winter, while adenovirus was observed throughout the year. **Conclusion:** In this study, it was shown that rotavirus and adenovirus were more significant causes of gastroenteritis, especially in winter among 0-5-year old group. It must also be stated that rapid and effective diagnostic methods are important to prevent unnecessary antibiotic usage, and to reduce the development of antibacterial resistance.

Key Words: Rotavirus; adenoviridae; gastroenteritis

ÖZET Amaç: Özellikle çocukluk çağında, mortalitenin en önemli nedenlerinden biri enfeksiyöz ishallerdir. Türkiyede epidemiyolojisi iyi bilinmeyen rotavirüs ve adenovirüs etkenlerine bağlı meydana gelen gastroenterit olgularının çeşitli yaş grupları, aylara ve yıllara göre dağılımını belirlemeyi amaçladık. **Gereç ve Yöntemler:** Ocak 2005 ve Haziran 2008 tarihleri arasında hastanemizin çeşitli kliniklerine başvuran ve akut gastroenterit tanısı alan 2,962 hastanın dışkı numunelerinde, rotavirüs ve adenovirüs antijenleri immunokromatografik yöntem ile araştırıldı. **Bulgular:** İkibindokuz yüzaltmışiki dışkı örneğinin 605'inde viral antijenler saptandı. Pozitif örneklerin 483'ü rotavirüs, 77'si adenovirüs yönünden pozitif olarak bulundu. Kırkbeş örnekte her iki virüs için birlikte pozitiflik saptandı. Viral gastroenterit olgularına (rota ve adenovirüs) en sık 0-5 yaş grubunda rastlanmakta olup, rotavirüs daha çok kış aylarında, adenovirüs ise bütün yıl boyunca görülmektedir. **Sonuç:** Bölgemizde özellikle kış aylarında ve 0-5 yaş grubunda gelişen gastroenteritlerde rota ve adenovirüsün önemli bir etken olduğu görülmektedir. Hızlı ve geçerli (yüksek duyarlılık ve özgüllükte) tanı yöntemleri antibiyotik kullanımının önlenmesi ile beraber antibakteriyel direnç gelişiminin azaltılması yönünden önemlidir.

Anahtar Kelimeler: Rotavirüs; adenovirus; gastroenterit

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Acute gastroenteritis appears due to enteropathogens such as viral, bacterial, or protozoal microorganisms. Rotavirus and adenovirus are among the most important factors causing severe gastroente-

ritis in adults and children.[1] Outcome of diseases caused by rotavirus and adenovirus is more serious than other pathogens; causing gastroenteritis leading to severe diarrhea, vomiting, fever, and rapid dehydration. Although these viruses are transmitted by human-to-human interaction by oral-fecal route and environmental routes in general, people are not sufficiently informed about rotavirus and adenovirus in developing countries. About two million cases of diarrheas caused by rotavirus are estimated to be hospitalized every year throughout the world. Annual rotavirus mortality rate is 600.000 children per year, most of them being in developing countries.[2,3] Although bacterial pathogens are routinely investigated in gastroenteritis, rotavirus and adenovirus are not yet adequately investigated. On the other hand, empirical antibiotic treatment is used frequently for, gastroenteritis resulting with the development of antibacterial resistance. Thus, it is recommended to determine rotavirus and adenovirus antigens rapidly in stools samples by immunochromatographic tests to prevent the problems mentioned above.[4]

The aim of this retrospective study is to characterize the frequency of rotaviruses and adenoviruses presented in Ankara as a function of age group, with particular attention to pediatric patients.

MATERIAL AND METHODS

Stool samples of 2,962 patients with ages ranging between 0-94 years-old admitted to our hospital with the diagnosis of acute gastroenteritis between January 2005 and June 2008 were analyzed for rotavirus and adenovirus antigens by immunochromatographic method (CerTest Biotec, Spain). This test is a qualitative immunochromatographic assay for the determination of rotavirus and adenovirus in feces samples. According to routine procedures and considering the directives of the physician, the stool samples from all gastroenteritis patients were collected in clean containers and transferred to our laboratory.

The stool samples, which were watery and did not contain leucocyte, were immediately transferred to the laboratory, examined by direct microscopy, and then cultured. The immunochromatographic method was also applied for the detection of the viral antigens. This method was carried out according to the procedure mentioned by the manufacturer.

Sensitivity and specificity of the kit have been reported as >99%, 98% for rotavirus, and 99% and >99% for adenovirus, respectively.

All the data were collected retrospectively from the recorded data on the Hospital Information System (HIS). This study was conducted in the last six months of 2008. The time period of the study includes the years of 2005, 2006, 2007, and the first period of 2008.

Data of the study were analyzed by SPSS 15.0 statistical package program. Frequencies and percentage distributions, means, standard deviation, median (50% value) values, first (25% value) and third quartiles (50% value) in this study were evaluated as continuous variables. Statistical significances of the differences between bivariate analyses were evaluated with Chi-square test, t-test, or ANNOVA accordingly.

RESULTS

General data obtained from the study is summarized in Table 1. The average patient age was calculated as 13.4 ± 18.0 (lower limit=0, upper limit=94). The group consisted of 47.6% and 52.4% female and male patients, respectively. As a result of the study, it was determined that out of 2,962 patients, 483 (16.3%) contained rotavirus antigen, 77 (2.6 %) patients were positive for adenovirus antigens and 45 (1.5 %) of them included both antigens (Table 1).

There was no significant difference statistically for the positivity of rotavirus and adenovirus among male and female patients (respectively p= 0.085 and p= 0.126).

185 stool samples, among the total of 2.962, were collected in 2005; 557 of them were collected in 2006 while the collected sample number was 1404 in 2007. Lastly, 816 samples were collected in the first six months of 2008. Years of collection and

TABLE 1: Distribution of existence of rotavirus and adenovirus antigens in stool samples according to gender in the years between 2005-2008.

	Number of Samples	Rotavirus* % (n)	Adenovirus* % (n)	Rota and Adeno* % (n)
Male	1553	8 (236)	1.6 (47)	0.6 (18)
Female	1409	8.3 (247)	1 (30)	0.9 (27)
Total	**2.962**	**16.3 (483)**	**2.6 (77)**	**1.52 (45)**

*Percentage (%)= (agent X100) / total samples.

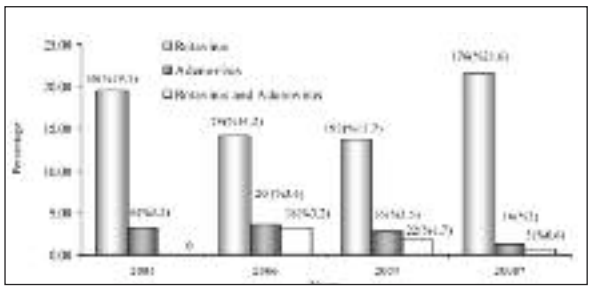


FIGURE 1: Distribution of rotavirus and adenovirus antigens in stool samples according to years (*2008 data cover January-June).

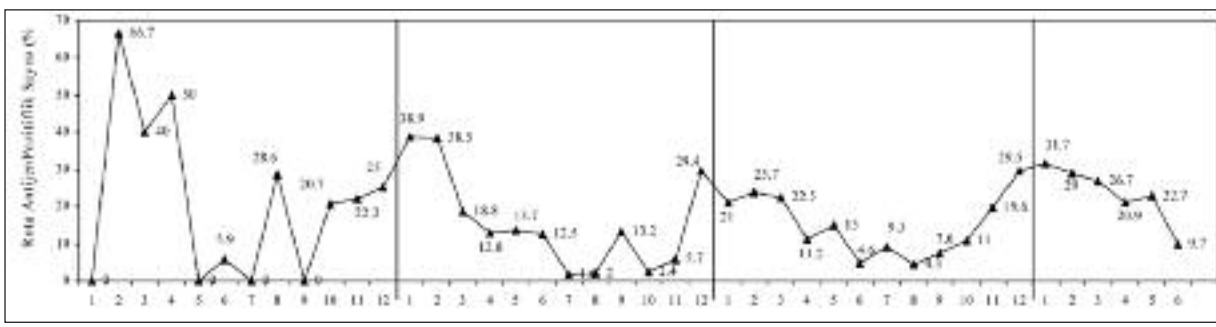
the distribution of rotavirus and adenovirus antigens in stool samples according to years are summarized in Figure 1.

Since the data collected in 2008 do not represent the whole year, distribution of samples and laboratory results according to months have been evaluated between 2005-2007.Thus, it can be concluded that samples were mostly sent to the laboratory in the months of September (14.8%) and October (12.6%). On the other hand, the highest rotavirus positivity was detected in February (29.6%) while December (28.7%), January (26.5%), and March (22.9%) collections also showed high incidence (Figure 2a). This result shows a statistically significant level (p= 0.001).

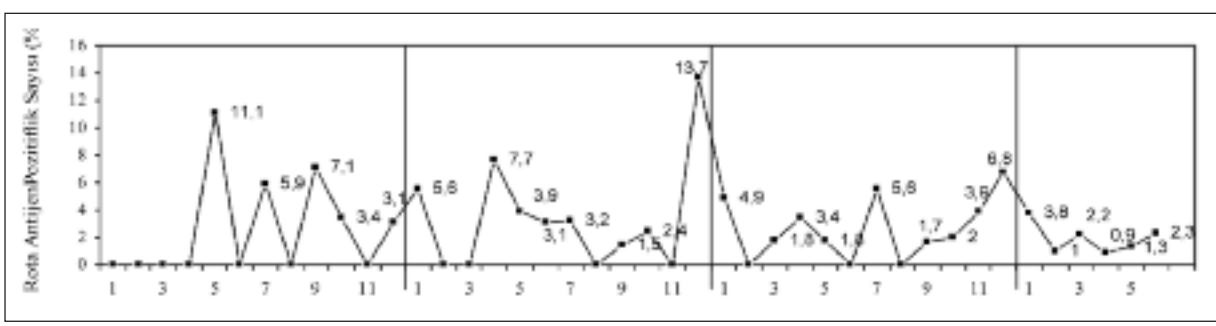
The distribution months of adenovirus positivity, on the other hand, is shown in Figure 2b, however, due to insufficient numbers, it could not be analyzed statistically.

The average age for rotavirus antigen positive patients was 10.3 ± 17.9 years whereas this average was 13.0 ± 17.3 years for antigen negative patients. This result shows a statistically significant level (p= 0.01).

Out of 2.962 samples, 56.3% (n= 1669), belongs to the 0-5 age range. Of total 483 rotavirus



a. Rrotavirus.



b. adenovirus

FIGURE 2: Distribution of **a.** rotavirus and **b.** adenovirus according to months between January 2005 and June-2008.

positive results, 337 (69.8%) are included in this age range. Likewise, 64 (83.1%) of total 77 positive adenovirus results, and 28 (62.2%) of 45 combined antigen positive cases, belong to the same age range.

The comparison of age range of 0-5 years and other age groups can be seen in Figure 3. Rotavirus positivity is found to be usually higher than adenovirus in all age ranges (Figure 3). When the results of age range 0-5 are compared with the other groups, the same finding is found especially for 1-4 years age groups.

The highest positivity is observed in age 5 (8.1%) when for the adenovirus positive cases. Moreover, the highest combined antigen positive group belongs to age 3 (3.3%) (Figure 3). It is obvious that rotavirus positivity is higher than adenovirus positivity in all age groups when the results in Figure 3 are considered.

DISCUSSION

It has been known for a long time that viruses play an important role in the etiology of infectious diarrhea. Moreover, rotaviruses and enteric adenoviruses are significant factors in viral enteritis in infants and children.[5,6] Rotaviruses appearing in temperate climates, generally between autumn and spring, are the major agents for childhood diarrheas .[7] It was reported that rotavirus was responsible for 11-71% and adenoviruses for 2-22% of viral gastroenteritis cases in various countries.[8,9]

In the studies conducted in Turkey, it has been reported that rotavirus positivity was between 9.8% and 39.8%,[10,11] and this rate was 7.8-10% for adenovirus positivity.[4] In this study, these values were 16.2% (483) and 2.3% (77) for rotavirus and adenovirus, respectively. It can be concluded that weather conditions and the epidemiological factors differ according to the study region. Although it was enteritis was stated that the frequency of adenovirus enteric are higher in male patients in different reports, some others suggested that the gender did not play any role in the incidence of viral enteritis.[12-17] Similarly, no statistically significant difference was detected between female and male patients for the rotavirus and adenovirus positivites (p= 0.085 and p= 0.126, respectively) in this research.

It was reported in previous studies that rotavirus positivity was always higher than that of adenovirus. Only exception is the study that has been carried out in Guetamala. It was detected that adenovirus cases were three fold more than the cases of rotavirus, and this situation was attributed to the climatic differences.[14]

In previous studies conducted in our country, adenovirus frequency was reported only between 4.717.8% for 5 years of age while this

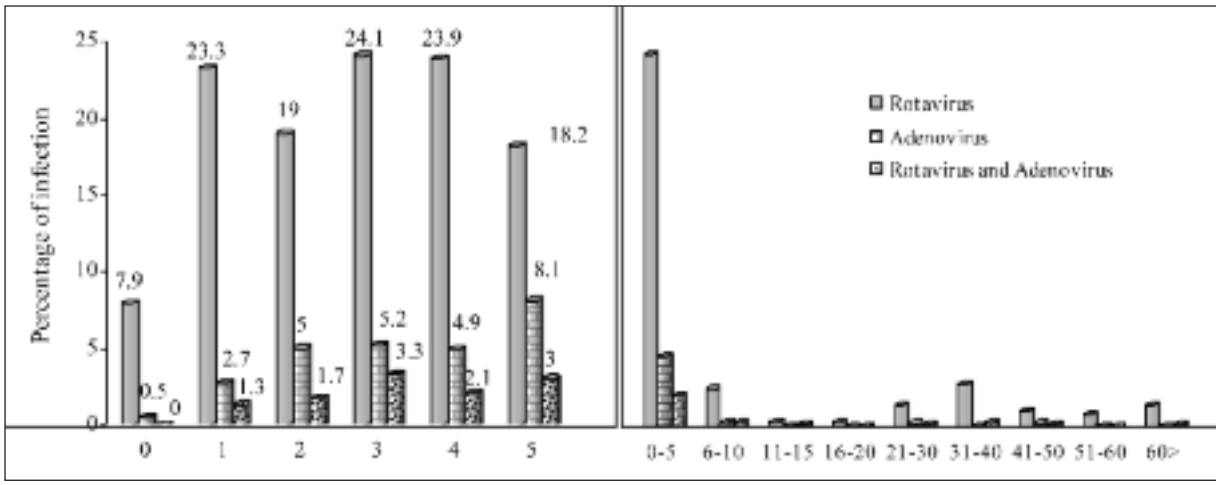


FIGURE 3: Rotavirus, adenovirus, and rota-adenovirus distribution of 0-5 years and other age groups between January 2005 and June-2008.

value was 7.1-33.3% for rotavirus frequency.[18,20] On the contrary, some investigators have reported rotavirus as the common virus among the most frequently observed, especially in 1-3 year old group.[12-

In this present research, the data revealed that highest rotavirus positivity (16.3%) was observed in ages between 0-5, highest number of cases occurring in 3 year olds. Moreover, it was detected that enteric adenovirus antigens are more common in age group of 5 (8.1%). For combined antigen positive group, it can be stated that highest positivity was observed in age 3 (3.3%) for this research while it was reported as between 1.3-6% in studies of similar content.[18,21] Furthermore, association of rotavirus and adenovirus in this study was found to be as 1.52%.

The frequency of infectious diarrhea cases show variability according to the season and geographic region.[22,23] It has been reported previously that rotavirus gastroenteritis is most frequent during winter and at the beginning of spring while adenovirus is observed throughout the year.[8,10,14,22] In Turkey, rotavirus is highest in January, April, and February.[19,25,26]

Our data are similar to previous reports.[8,10,14,24] It can be concluded that the distribution of rotavirus and adenovirus throughout the seasons may change due to the effect of global warming in the future.

In conclusion, this study revealed that rotaviruses and adenoviruses are important causes for gastroenteritis that developed especially during winter in pediatric age group in the study region. Further researches are required for determining presence of viral factors and displaying their significance for gastroenteritis that is widespread in Turkey, especially in some regions. Routine investigations for rotavirus and adenovirus antigens would provide the rapid diagnosis in the region.

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REFERENCES

1. Walsh JA, Warren KS. Selective primary health care: an interim strategy for disease control in developing countries. N Engl J Med 1979;301(18):967-74.
2. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006;12(2):304-6.
3. Bresee J, Fang ZY, Wang B, Nelson EA, Tam J, Soenarto Y, et al. Asian Rotavirus Surveillance Network. First report from the Asian Rotavirus Surveillance Network. Emerg Infect Dis 2004;10(6):988-95.
4. Nazik H, İlktaç M, Öngen B.[Investigation of frequency of rotavirus in childhood acute gastroenteritis]. Ankem Derg 2006;20(4):233-5.
5. Saderi H, Roustai MH, Sabahi F, Sadeghizadeh M, Owlia P, De Jong JC. Incidence of enteric adenovirus gastroenteritis in Iranian children. J Clin Virol 2002;24(1-2):1-5.
6. Şimşek Y, Bostancı İ, Bozdayı G, Öner N, Kamruddin A, Rota S, et al. [Frequency and serotype features of rotavirus in 0-5 age children with acute gastroenteritis]. Turkiye Klinikleri J Pediatr 2007;16(3):165-70.
7. Ceyhan M. [Viral gastroenteritis]. Katkı Pediatri Derg 2000;21(30):34-64.
8. Sánchez-Fauquier A, Montero V, Moreno S, Solé M, Colomina J, Iturriza-Gomara M, et al. Human rotavirus G9 and G3 as major cause of diarrhea in hospitalized children, Spain. Emerg Infect Dis 2006;12(10):1536-41.
9. Román E, Wilhelmi I, Colomina J, Villar J, Cilleruelo ML, Nebreda V, et al. Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children. J Med Microbiol 2003;52(Pt 5):435-40.
10. Kurugöl Z, Geylani S, Karaca Y, Umay F, Erensoy S, Vardar F, et al. Rotavirus gastroenteritis among children under five years of age in Izmir, Turkey. Turk J Pediatr 2003; 45(4):290-4.
11. Çam H, Gümüş A. [Assessment of rotavirus frequency in cases with acute gastroenteritis]. Hipokrat Pediatri Dergisi 2003;3:127-30.
12. Doğan N, Akgün Y. [Presence of Rotavirus in 0-6 age gastroenteritis]. Turkish J Infec 1998;12(4):493-5.
13. Karslıgil T, Kılıç İH, Balcı İ. [Rotavirus gastroenteritis in children between 0-6 years of age and it's impact on lactose intolerance]. J Turkish Microbiol Soc 2003;33(2):137-42.
14. Cruz JR, Cáceres P, Cano F, Flores J, Bartlett A, Torún B. Adenovirus types 40 and 41 and rotaviruses associated with diarrhea in children from Guatemala. J Clin Microbiol 1990;28(8):1780-4.
15. Coşkun Ş, Önal O, Keskin M. [Rotavirus in acute diarrhea cases]. Turkish J Infec 1993;7(3-4): 273-5.
16. Huilan S, Zhen LG, Mathan MM, Mathew MM, Olarte J, Espejo R, et al. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. Bull World Health Organ 1991;69(5):549-55.
17. Parashar UD, HolmanRC, Clarke MJ, Bresee JS, Glass R. Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. J Infect Dis 1998;177(1):13-7.
18. Schoenemann W. [Significance of adenovirus infections in infancy and early childhood]. Monatsschr Kinderheilkd 1988;136(10):680-5.

19. Gül M, Garipardıç M, Çıragil P, Aral M, Karabiber H, Güler L. [Investigation of rotavirus and adenovirus types 40/41 in children with gastroenteritis between 0-5 years of age]. Ankem Derg 2005;19(2):64-7.

20. Altındiş M, Beştepe G, Çeri A, Yavru S, Kalaycı R. [Frequency of rotavirus and enteric adenovirus infection in children with acute gastroenteritis]. SDÜ Tıp Fak Derg 2008; 15(2):17-20.

21. Hoshino T, Hosokawa N, Yanai M, Kumasaka K, Kawano K. [A study of serum mitochondrial enzymes (mCK, mAST, mMDH) in rotavirus and adenovirus gastroenteritis in pediatric pa¬tients]. Rinsho Byori 2001;49(11): 1157-61.

22. Giordano MO, Ferreyra LJ, Isa MB, Martinez LC, Yudowsky SI, Nates SV. The epidemiology of acute viral gastroenteritis in hospitalized children in Cordoba City, Argentina: an insight of disease burden. Rev Inst Med Trop Sao Paulo 2001;43(4):193-7.

23. Yıldırmak Y, Tanyer G, Dallar Y, Serdaroğlu A. [The clinical and epidemiological features in acute gastroenteritis due to rotavirus and other ethiologic agents in infants]. Turkiye Klinikleri J Pediatr 1992;1(1):1-6.

24. Akıncı N, Ercan Erener T, Yalman N, Eren A, Severge B, Ercan D. [Adenovirus and Rotavirus in Children with Acute Gastroenteritis]. J Pediatric Infection 2007;1(3):98-101.

25. Durmaz ÇB, Hatipoğlu S, Önal Sönmez E, Gündüz A, Seber E. [Rotavirus infections in gastroenteritis cases that between 0-5 years old]. J Turkish Microbiol Soc 2001;31(3-4):263-5.

26. Turgut M. [Viral gastroenteritis]. Turkiye Klinikleri J Pediatr Sci 2004;2(3):260-4.