Le Infezioni in Medicina, n. 4, 261-269, 2013

Articoli originali

Original articles

Annual report on norovirus in children with acute gastroenteritis in 2009 and their genotypes in Turkey

Rapporto annuale sull'incidenza dell'infezione da norovirus, e relativa genotipizzazione, in bambini con gastroeneterite acuta nel 2009 in Turchia

Defne Çöl[1], Suat Biçer[1], Gülay Çiler Erdağ[1], Tuba Giray[1], Yeşim Gürol[2], Gülden Yılmaz[2], Öznur Küçük[1], Ayça Vitrinel[1]

[1]Department of Child Health and Pediatrics, Faculty of Medicine, Yeditepe University, İstanbul, Turkey;
[2]Department of Medical Microbiology, Faculty of Medicine, Yeditepe University, İstanbul, Turkey

INTRODUCTION

Diarrheal disorders in children account for a large proportion (18%) of childhood deaths, with an estimated 1.5 million deaths per year globally, making it the second most common cause of child deaths worldwide. The World Health Organization and UNICEF estimate that almost 2.5 billion episodes of diarrhea occur annually in children under 5 years age in developing countries, with more than 80% of the episodes occurring in Africa and South Asia (46% and 38%, respectively) [1]. The etiology and clinical course may vary depending on age, season and demographics. In developing countries, the morbidity and mortality associated with acute infectious diarrhea is higher in children under 5 years of age [1]. Acute gastroenteritis is caused by viruses, bacteria, and protozoa [1, 2]. Viruses affect all age groups, can cause sporadic or endemic infections, and are responsible for most of acute gastroenteritis of undetermined etiology [1]. Norovirus is one of the leading cause of acute gastroenteritis in infants, children and adults [3]. Viral gastroenteritis usually occurs in cold and rainy months worldwide, with transmission being mainly through the fecal-oral route [1-3]. The epidemiology of norovirus patients in Turkey is not well known because of laboratory limitations and underreporting. This study was undertaken between January 2009 and January 2010 at Yeditepe University Hospital, in Istanbul, Turkey.

Phylogenetically, genus norovirus is classified in *Caliciviridae* and includes at least five genogroups (GI, GII, GIII, GIV, GV) with extensive genetic diversity [4, 5]. Norovirus GI, GII and GIV may cause infections in human hosts [6]. Most of the infections due to norovirus is caused by strains in GII (range 75%-100%) and GI [7]. Especially strains in GII.4 genotype have high pandemic potential due to their antigenic variation [8, 9].

The diagnosis of norovirus is possible by antigen detection with different methods. Immunochromatography is a reliable tool for the detection of norovirus [10, 11]. Polymerase chain reaction (PCR) is increasingly used to detect genetic material and genogroups of enteric viruses [12]. Mostly used basic, rapid and cheap diagnostic methods for the detection with high sensitivity and specificity on the norovirus antigen are enzyme immunoassay (ELISA) and immunochromatography. However, compared to antigen tests, molecular techniques are more sensitive, more expensive and time consuming [12].

Corresponding author
Suat Biçer
E-mail: suat.bicer@yeditepe.edu.tr

The objective of this study was to determine the incidence, seasonal distribution and clinical characteristics of norovirus in children with acute gastroenteritis and to analyze the genogroups of norovirus.

MATERIALS AND METHODS

Study design

The patients with acute gastroenteritis who were admitted to the pediatric emergency, pediatric wards and outpatient clinics of Yeditepe University Hospital in 2009 were evaluated retrospectively. All patients aged 3 months - 16 years, with norovirus infection confirmed by immunochromatography and/or multiplex PCR, and with neither concomitant diseases nor underlying disorders were eligible for inclusion. Patients with mixed intestinal infections with bacteria or parasites, concomitant diseases (e.g., bronchiolitis, acute upper and lower respiratory tract infections, or urinary tract infection), or any other underlying disorders were excluded. The demographical characteristics, clinical signs, laboratory findings and symptoms, including vomiting, diarrhea, fever, dehydration and malaise of patients with norovirus were evaluated in this study. Patients in whom coinfection was detected and whose clinical and/or treatment findings were not obtained from medical records were excluded from clinical analysis. Unmeasurable or poorly-defined symptoms in young children (e.g., abdominal pain, headache, myalgia, nausea) were not evaluated.

Acute diarrhea was defined as at least 3 looser stools within a 24-hour period or an episode of forceful vomiting with any loose stools lasting less than 14 days. Diarrhea lasting more than 2 weeks or history of noninfectious gastrointestinal disease were accepted as exclusion criteria. Fresh stool samples were used to detect the agent. The timing of laboratory samples with more than 1 hour was excluded. The samples were transferred to the microbiology laboratory within 10 minutes. Microscopic examination of stools and viral antigen tests were carried out within an hour. Stool samples were stored at -20°C in microbiology laboratory. Enteric viruses, including norovirus, were studied with the immunochromatographic assay method (Rida Quick norovirus, r-biopharm, Germany). All patients were tested also for bacteria and parasites to detect coinfections. Stool samples were delivered at the Yeditepe University Medical Microbiology Laboratory under convenient conditions to be evaluated. Enteric viruses (adenovirus, norovirus, and rotavirus), stool microscopy, and culture were tested routinely in patients with acute diarrhea.

The demographical and clinical data were obtained from hospital medical records. Seasonal divergence within a year and quantitative distribution were analysed. Norovirus infections were set for age groups as first 2 years, 2-5 years, and older than 5 years. Norovirus was examined in 520 stool samples. Symptoms, clinical characteristics, treatment methods and laboratory findings were evaluated in patients who had positive results only for this virus.

In our study some randomly selected subsets of antigen positive samples (n = 28) were analyzed for genogroups (i.e., genogroup I or genogroup II) with the multiplex-PCR method using the Seeplex Diarrhea ACE Detection kit (Seegene, Korea). Norovirus genogroup II was detected in 100% (28/28) of the samples.

Statistical analysis

The statistical analysis was made using the SPSS programme version 18 (SPSS, Chicago, IL). For the comparison of seasonal distribution, clinical manifestations, and laboratory results among patients, we divided subjects into two subgroups according to the year. The results were analysed using Student t test, with chi-square tests for group comparison. The results were presented as values with or without percentage. Data were considered as statistically significant if $p<0.05$.

RESULTS

A total of 520 patients (mean age 30.4 months) were enrolled in the study. Norovirus was detected in 50 (9.6%) samples out of 520, in children under 17 years of age who had been admitted with acute gastroenteritis. Incidence of norovirus in patients 3-24 months of age was 13.7%. Analysis of the dataset according to age groups shows that norovirus was mostly detected in the first 24 months (50% of all patients) ($p<0.05$). The age distribution for norovirus positive patients was 50% (n=25) for patients under 24 months and 38% (n=19) in those under 5 years. The age distribution of the patients is shown in Table 1.

Coinfections were present in 4 patients (0.8%).

There was virus-virus coinfection in 4 patients. In 3 patients norovirus and adenovirus, and in 1 patient norovirus and rotavirus were found together as a coinfection (Table 1). These agents were diagnosed by immunochromatography.
Norovirus was most frequently seen in February, May, July and September, while it was not detected at all in October and it was rarely seen on other months (Figure 1).

Clinical characteristics of norovirus infection were analyzed in the 44 patients positive only for norovirus (Table 2). In six patients, clinical findings were not analysed due to coinfections (n = 4) and lack of medical records (n = 2). In the clinical presentation of infection, all patients with norovirus presented acute diarrhea, while vomiting was seen in 42/44 (95.5%) patients. Other symptoms and clinical findings were re-

Table 1 - Distribution of norovirus according to age groups.

Age groups	*No. pts investigated for norovirus*	*Patients positive for norovirus N (% of all investigated patients)*	*Coinfections*
0-5 years:	412	44 (10.7%)	3[§]
0-24 months	189	25 (13.2%)*	3
25-60 months	223	19 (8.5%)	0
6-16 years	108	6 (5.5%)	1[#]
Total	520	50 (9.6%)	4

[§](norovirus + adenovirus); [#](norovirus + rotavirus); *($p<0.05$)

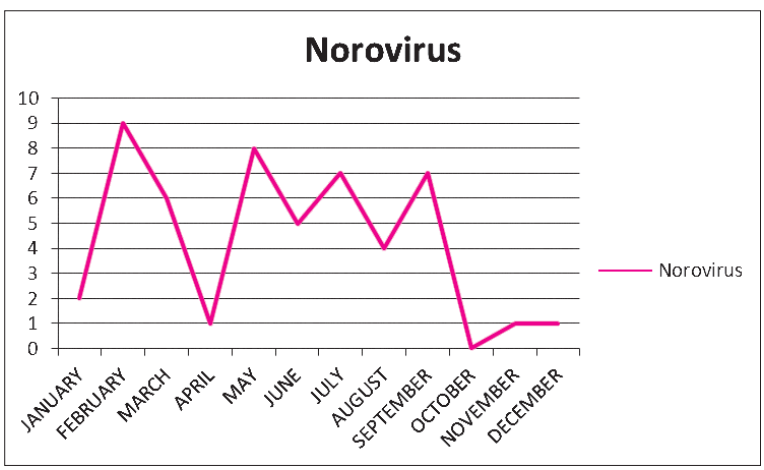

Figure 1 - Seasonal distribution of norovirus.

Table 2 - Symptoms/clinical findings of patients with norovirus infection (n=44).

Symptoms/clinical findings	*N (%)*	*3-24 months N*	*25-60 months N*	*6-16 years N*	*P*
Diarrhea	44 (100%)	20	18	6	<0.05
Vomiting	42 (95.5%)	20	17	5	<0.05
Decreased feeding	38 (86.4%)	17	16	5	<0.05
Malaise	37 (84%)	16	16	5	<0.05
Dehydration	26 (59%)	11	11	4	<0.05
Fever (>38°C)	11 (25%)	7	3	1	<0.05
Total	44*	20	18	6	<0.05

*Patients with coinfections and lack of medical records were excluded from clinical analysis.

Table 3 - Laboratory findings in some patients with norovirus infection.

Laboratory parameters	*N (%)*[a]	*Mean ± SD*	*Median*	*Range*
Sodium (mg/dL)	22 (4.23%)	138.18±2.60	138	134-143
BUN (mg/dL)	19 (3.65%)	18.73±4.90	18	12-32
Glucose (mg/dL)	13 (2.5%)	96.61±21.17	99	55-125
Neutrophilia (/mm3)	23 (4.4%)	13732±7068.57	12360	600-32410
CRP (mg/L)	23 (4.4%)	8.66±4.82	9.65	0.3-20.15
Urine density	25 (4.8%)	1025.6±5.35	1025	1010-1030

[a]Number and percent of investigated patients.

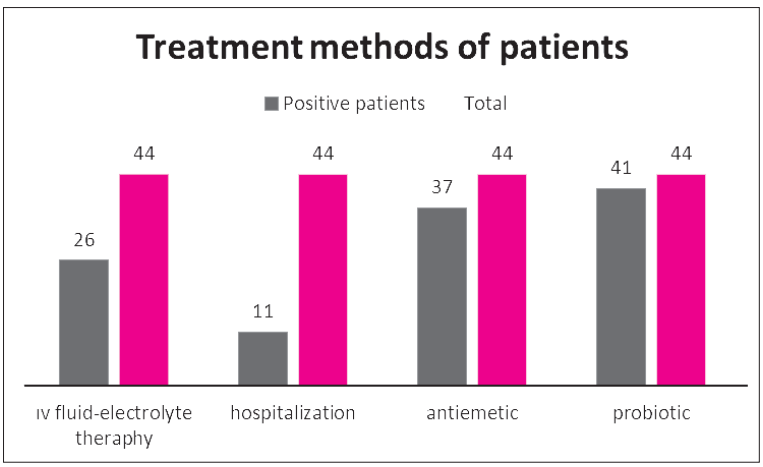

Figure 2 - Treatment methods of patients with norovirus.

duced feeding (n =38; 86.4%), malaise (n =37; 84%), mild to severe dehydration (n =26; 59%), and fever (n =11; 25%) (Table 2). There were no statistically significant differences for symptoms and clinical findings among age groups (p>0.05).

The laboratory findings were evaluated in various numbers (range: 13 - 25) of patients. Sodium levels were evaluated in 22 patients, hyponatremia was detected in only one patient (4.5%). Other electrolyte disorders (i.e. hypernatremia, hypokalemia, hyperkalemia, hypochloremia, hyperchloremia) were not detected any of the patients. Elevated blood urea nitrogen (7 patients, 7/19, 36.8%), hyperglycemia (3 patients, 3/13, 23%), leukocytosis (6 patients, 6/22, 27.3%), neutrophilia (13 patients, 13/23, 56.5%), elevated CRP (19 patients, 19/23, 82.6%), elevated (>1015) urine density (23 patients, 23/25, 92%), and ketonuria (20 patients, 20/25, 80%) were detected in some patients (Table 3). There were no statistical differences in the laboratory findings among age groups (p>0.05).

Treatment methods are shown in Figure 2. Antiemetic drugs and intravenous fluid-electrolyte theraphy were required in 37 (84%) and 26 (59%) patients, respectively. Some patients were hospitalized (n =11, 25%). Mean hospitalization length was 23.12 (range: 6-42) hours. Probiotics were used in 41 patients (93.2%). There were no statistical difference for treatment methods among age groups (p>0.05).

DISCUSSION

The determination of the etiology of acute gastroenteritis is important for the treatment and prognosis [1, 13-16]. The detection rate of viruses and knowledge of disease epidemiology improved due to the developments of immunochromatography, enzyme immunoassays and real time PCR assays for viruses. The detection rate of norovirus reached between 4.5% and 48.4%, depending on the methodological differences and the age of the patients [18]. GI, GII, and GIV genogroups of norovirus were identified in

patients with acute gastroenteritis. Some strains may have high pandemic potential due to antigenic variation; Bull et al. and Lindesmith et al. documented the high pandemic potential in GII.4 genotype [8, 9]. In this study, the incidence of norovirus was 10.7% in patients up to 5 years, and 13.7% up to 24 months of age. Our results have confirmed that norovirus is one of the most common viral agent in children (≤5 years) with acute gastroenteritis as shown in other studies [17, 19-21]. As reported previously, rotavirus was the most frequently detected viral agent in children [17, 21-24]. Annual seasonal distribution of enteric viruses varies with climate worldwide. Norovirus was detected throughout the year, but the rate of isolation was higher in winter to spring [17, 25, 26]. As this study noted, norovirus was the most dominant viral agent in hottest months of the year (July, August and September).

Clinically, it may be difficult to differentiate viral gastroenteritis from others, so methods like electron microscopy, culture or antigen detection through enzyme immunoassay or latex agglutination and RT-PCR are necessary to confirm viral etiology [13, 15, 17, 27, 28]. Electron microscopy is a fast and reliable technique, but it is not widely used in practice [28]. RT-PCR is more sensitive but not rapid method to detect viruses. Since the total genome of norovirus has been sequenced, norovirus may be detected by more sensitive molecular methods such as nested RT-PCR [29]. This method is more sensitive than conventional RT-PCR [12]. Norovirus was detected in 6.3% of fecal samples using nested RT-PCR [29]. Enzyme immunoassay and immunochromatography methods are basic and widely used diagnostic tools for norovirus antigen detection with high sensitivity and specificity [10]. The sensitivity and specificity of ELISA is between 59% and 76.9% and between 73.1% and 85.9%, respectively [11]. Wu et al. suggest that the incidence of norovirus detection may be raised when enzyme immunoassays are combined with the more sensitive RT-PCR [30].

Lately immunochromatography method has gained popularity since the results are readily available in 5-10 minutes, may be run with small amount of sample and has high sensitivity (93-100%) [15, 27]. These tests may be unreliable in newborns and patients with underlying intestinal problems because of false positivity rates [27].

It is thought that norovirus is one of the major pathogens leading to diarrheal outbreaks and causes symptomatic infections in older children and adults. However, some studies have demonstrated that norovirus also has an impact on children under 2 years, which is consistent with the findings of the current study [31]. In the present study norovirus infections were mostly seen in patients under two years of age; other studies showed positivity rates of norovirus infections of 17.3% in Spain, and 14.6% in Taiwan during the first 5 years [32, 26]. In Taiwan, norovirus were more commonly seen at 0-36 months (84.7%) and 0-24 months (67.4%) [26]. In our study, among the 50 norovirus-positive patients, 49% were up to 2 year of age, and 39.2% were between 2 and 5 years of age.

Coinfections may also be seen, mostly dual viral infections rather than bacterial - viral coinfections [3, 15, 17, 33]. In our study, the rate of coinfections (0.8%) was low in comparation to other studies reported in other European countries. Our study has shown coinfections of rotavirus-norovirus, adenovirus-norovirus gastroenteritis.

Vomiting, diarrhea, abdominal pain, fever, anorexia, malaise, dehydration are the most commonly seen symptoms of acute gastroenteritis. Palumbo et al. have shown that diarrhea (93%) and vomiting (87%) were the mostly presented symptoms in patients with acute diarrhea [16]. There were no norovirus positive patients in their study. Clinical studies have shown that most patients with norovirus manifested as vomiting (94%) and diarrhea (81%). Narkeviciute et al. have shown that norovirus infection was presented with more intense vomiting but less severe diarrhea; patients with norovirus were presented with vomiting (94%), diarrhea (81%) and fever (66%) in their research [34]. Vomiting is the most prominent feature of norovirus infection in several reports [35-38]. Vomiting has been reported by many authors as a mostly remarkable feature of norovirus infection [32, 35-38]. Götz et al. have shown in their study that 40% of patients had vomiting without diarrhea [38]. Diarrhea was absent in 19% of the patients according to the reports of Narkeviciute et al. [34]. In our study, vomiting was described as the most prominent feature of norovirus infection. However, only diarrheal patients were evaluated in our study. For this reason, patients who had vomiting without diarrhea were not included in our report. In the clinical presentation of our patients, diarrhea and vomiting were mostly seen in norovirus gastroenteritis. In

our clinical observations some patients with acute gastroenteritis manifested vomiting with or without diarrhea. To facilitate the diagnosis of norovirus in patients presenting only vomiting, without diarrhea, norovirus may be detected in vomit samples using PCR [39].

Fever was one of the mostly encountered symptoms in acute gastroenteritis including norovirus. However, high fever was reported less commonly compared to rotavirus gastroenteritis in various studies [26, 32, 34]. Yang et al. reported that patients with norovirus gastroenteritis were presented with a low fever, no fever, and a longer duration of vomiting [26]. Narkeviciute et al. showed that fever was presented in 66% of patients with norovirus. Fever was less common (25%) in our patients compared with other reports on norovirus infections. In other reports, fever during norovirus infection was present in 24% to 48.3% of patients, and high fever (>38°C) in just 21% of the patients [34, 36, 38].

Malaise was observed in 84% of our studied patients. In the study of Palumbo et al. on diarrhea in children this symptom was observed in 53.4% but it must be said that no patient had norovirus infection [16].

Dehydration (mild to severe) was diagnosed in 59% of the patients and was low compared to the study of Narkeviciute et al. (76%); however, only hospitalized patients were evaluated in this report [34]. Hospitalization ratio was reported as 14.6% by Yang et al. [26]. In another report, dehydration was present only in two patients (6.9%), whereas intravenous fluid-electrolyte therapy was required in 12 patients (41.4%) because of persistent vomiting [32]. By contrast, in some studies, due to milder symptoms, hospitalization was needed for only 1.4% of the patients [37]. Intravenous fluids and hospitalization were required in our patients with moderate and severe dehydration in 59% and 25% of the patients respectively.

Leukocytosis (27.3%) and neutrophilia (56.5%) were the most common laboratory findings in our patients. The differential diagnoses of leukocytosis involves physiologic responses to many infections including viruses. Norovirus infection is a viral disease; however, some studies demonstrated that mild to moderate elevated levels of CRP along with leukocytosis and neutrophilia are noted in patients with norovirus diarrhea [26, 30, 40]. For this reason, leukocytosis and neutrophilia may be related with vomiting and dehydration.

CONCLUSION

It is concluded that viruses were the major pathogens in all age groups. Norovirus is one of the most important etiologic agents of acute gastroenteritis in Turkish children especially in first 2 years of age. In 2009, the incidence of norovirus detection was high during late spring, summer and early autumn, whereas it was rare in other months and was not detected at all in October. Like other enteric viruses, diarrhea and vomiting were the mostly revealed symptoms in patients with norovirus. Antiemetic drugs, intravenous fluid-electrolyte therapy, and hospitalization in some patients, were required in norovirus diarrhea. Immunochromatography is a simple and fast test, the viral etiology can be quickly diagnosed with this method and this may help to predict the clinical course and avoid antibiotic usage. Molecular techniques (PCR) may enhance overall diagnostic efficacy for viral and bacterial pathogens, but this technique is expensive and time-consuming compared to immunochromatography. The present study suggests that a norovirus vaccination would be desirable as per rotavirus in order to decrease morbidity and hospitalization rates.

ACKNOWLEDGMENTS

We would like to thank Şilem Gündüz, Deniz Karadeniz, Burcu Öksüz and Şahap Aksaçlı (biologists at Yeditepe University Faculty of Medicine, Department of Medical Microbiology) for their excellent technical assistance in analyzing the virological tests, and Assistant Professor Çiğdem Kaspar MD fromYeditepe University Faculty of Medicine Department of Statistics for the statistical evaluations. We also would like to thank to Cahit Zereycan (writing consultant of Writing Center at Yeditepe University) for the revision of this paper in English.

Funding: This study was not funded.

Conflict of interest statement: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval: This study was ethically approved by local ethical committee of Yeditepe University Hospital (date: 02.06.2009).

Keywords: Acute gastroenteritis, child, immunochromatography, multiplex polymerase chain reaction, norovirus.

SUMMARY

In order to determine the incidence, seasonal distribution and clinical characteristics of norovirus in children and analyse the genogroups of norovirus, immunochromatography was used to detect the virus in stool samples. Randomly selected subsets of samples were analysed for genogroups with a multiplex polymerase chain reaction method. Seasonal distribution of norovirus, symptoms, physical and laboratory findings of patients and treatment models were evaluated retrospectively in 2009. In all, norovirus was examined in 520 stool samples.

The infection rate was 9.6% (50/520) among patients of acute gastroenteritis in 2009. The virus was mostly detected in the first 24 months of life (50%). Gastroenteritis with norovirus was most frequently found in February, May, July and September. The main symptoms were diarrhea (100%) and vomiting (95.5%). In some patients affected by norovirus infection higher urine density, ketonuria and high CRP levels were observed. Antiemetic drugs and intravenous fluid-electrolyte therapy were given to 37 (84%) and 26 (59%) of patients, respectively. Hospitalisation was required in 11 patients (25%). All the randomly selected 28 samples (100%) had norovirus genogroup II. In conclusion, norovirus (genogroup II) mostly affected children in the first two years of life and was more frequently observed in February, May, July and September of 2009. Diarrhea and vomiting were the most frequent symptoms. Antiemetic drugs, intravenous fluid-electrolyte therapy and hospitalisation were usually required in these patients.

RIASSUNTO

Il presente studio è stato condotto al fine di determinare l'incidenza, la distribuzione stagionale e le caratteristiche cliniche dell'infezione da norovirus in età infantile e analizzare i genotitpi virali. La rilevazione di norovirus nei campioni di feci è stata effettuata mediante immunocromatografia. L'analisi del genotipo virale è stata realizzata mediante PCR. Complessivamente, la ricerca del norovirus è stata eseguita in 520 campioni. L'incidenza di infezione da norovirus nei bambini affetti da gastroenterite acuta è risultata nel 2009 pari al 9,6% (50/520). Il maggior numero di casi (50%) sostenuti da norovirus è stato osservato in bambini di età inferiore a 24 mesi. I casi di gastroenterite da norovirus sono stati riscontrati con maggiore frequenza nei mesi di febbraio, maggio, luglio e settembre. I sintomi principali erano costituiti da diarrea (100%) e vomito (95%). In taluni pazienti affetti da infezione da norovirus sono stati osservati una maggiore densità delle urine, chetonuria e livelli elevati di PCR. Farmaci anti-emetici e soluzione fisiologica per via endovenosa sono stati somministrati a 37 (84%) e 26 (59%) pazienti, rispetttivamente. L'ospedalizzaizone si è resa necessaria in 11 pazienti (25%). Tutti i 28 campioni, selezionati in maniera casuale, sui quali è stata eseguita l'analisi genotipica hanno evidenziato la presenza di norovirus genogruppo II. In conclusione, nel 2009 il norovirus (genogruppo 2) ha colpito soprattutto i bambini nei primi 2 anni di vita ed è stato osservato con maggior frequenza nei mesi di febbraio, maggio, luglio e settembre. In tali pazienti, è stato generalmente necessario ricorrere a farmaci antiemetici, alla somministrazione per via endovenosa di liquidi ed elettroliti e all'ospedalizzazione.

REFERENCES

[1] Bhutta Z.A. Acute gastroenteritis in children. In: Nelson Textbook of Pediatrics, 19th ed (Kliegman R.M., Stanton B.F., St. Geme III J.W., Schor N.F., Behrman R.E. Eds) 2011, 1323-1339. Saunders Elsevier, Philadelphia.

[2] Hart C.A. Introduction to acute infective Diarrhea. In: Manson's tropical diseases, 21st ed (Cook C., Zumla A., Eds) 2003, 907-913. W.B. Saunders, London.

[3] Hart C.A., Cunliffe N.A. Diagnosis and causes of viral gastroenteritis. *Curr. Opin. Infect. Dis.* 9, 333–339, 1996.

[4] Zheng D.P., Ando T., Fankhauser R.L., Beard R.S., Glass R.I., Monroe S.S. Norovirus classification and proposed strain nomenclature. *Virology* 346, 312-323, 2006.

[5] Phan T.G., Kaneshi K., Ueda Y., et al. Genetic heterogeneity, evolution, and recombination in noroviruses. *J. Med. Virol.* 79, 1388-400, 2007.

[6] Glass R.I., Parashar U.D., Estes M.K. Norovirus gastroenteritis. *N. Engl. J. Med.* 361, 1776-1785, 2009.

[7] Patel M.M., Widdowson M.A., Glass R.I., Akazawa K., Vinjé J., Parashar U.D. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg. Infect. Dis.* 14, 1224-1231, 2008.

[8] Bull R.A., White P.A. Mechanisms of GII.4 norovirus evolution. *Trends Microbiol*. 19, 233-240, 2011.

[9] Lindesmith L.C., Donaldson E.F., Baric R.S. Norovirus GII.4 strain antigenic variation. *J. Virol.* 85, 231-242, 2011.
[10] Khamrin P., Nguyen T.A., Phan T.G., et al. Evaluation of immunochromatography and commercial enzyme-linked immunosorbent assay for rapid detection of norovirus antigen in stool samples. *J. Virol. Methods* 147, 360-363, 2008.
[11] Wilhelmi de Cal I., Revilla A., del Alamo J.M., et al. Evaluation of two commercial enzyme immunoassays for the detection of norovirus in faecal samples from hospitalised children with sporadic acute gastroenteritis. *Clin. Microbiol. Infect.* 13, 341-343, 2007.
[12] Dennehy P.H. Acute diarrheal disease in children: epidemiology, prevention, and treatment. *Infect. Dis. Clin. North Am.*19, 585-602, 2005.
[13] Gül M., Garipardıç M., Çiragil P., Aral M., Karabiber H., Güler I. 0-5 Yas Arası Gastroenteritli Çocuklarda Rotavirüs ve Adenovirüs Tip 40/41 Arastırılması. *ANKEM Derg*. 19, 64-67, 2005.
[14] Wilhelmi I., Roman E., Sánchez-Fauquier A. Viruses causing gastroenteritis. *Clin. Microbiol. Infect.* 9, 4, 247-262, 2003.
[15] Bass D.M. Rotavirus and other agents of viral gastroenteritis. In: Nelson Textbook of Pediatrics, 17th ed. (Behrman R.E., Kliegman R.M., Jenson H.B., Eds) 2004, 1081-1083. WB Saunders Co. Philadelphia.
[16] Palumbo E., Malorgio C., Siani A., Bonora G. Diarrhea in children: etiology and clinical aspects. *Infez Med.* 2, 95-99, 2009.
[17] Levidiotou S., Gartzonika C., Papaventsis D. et al. Viral agents of acute gastroenteritis in hospitalized children in Greece. *Clin. Microbiol. Infect.* 15, 586-598, 2009.
[18] Colomba C., Saporito L., Giammanco GM., et al. Norovirus and gastroenteritis in hospitalized children, Italy. *Emerg. Infect. Dis*. 13, 1389-1391, 2007.
[19] Liu C., Grillner L., Jonsson K., et al. Identification of viral agents associated with diarrhea in young children during a winter season in Beijing, China. *J. Clin. Virol*. 35, 69-72, 2006.
[20] Chikhi-Brachet R., Bon F., Toubiana L., et al. Virus diversity in a winter epidemic of acute diarrhea in France. *J. Clin. Microbiol.* 40, 4266-4272, 2002.
[21] Marie-Cardine A., Gourlain K., Mouterde O. et al. Epidemiology of acute viral gastroenteritis in children hospitalized in Rouen, France. *Clin. Infect. Dis.* 34, 1170-1178, 2002.
[22] Bon F., Fascia P., Dauvergne M., et al. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. *J. Clin. Microbiol*. 37, 3055-3058, 1999.
[23] Oh D.Y., Gaedicke G., Schreier E. Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. *J. Med. Virol*. 71, 82-93, 2003.
[24] Biçer S., Şahin Tunca G., Koncay B., et al. Incidence assessment of rotavirus and adenovirus associated acute gastroenteritis patients in early childhood. *Infez. Med*. 2, 113-119, 2011.
[25] Huha J.W., Kima W.H., Moona S.G., Leea J.B., Limb Y.H. Viral etiology and incidence associated with acute gastroenteritis in a 5-year survey in Gyeonggi province, South Korea. *J. Clin. Virol.* 44, 152-156, 2009.
[26] Yang S.Y, Hwang K.P., Wu F.T., et al. Epidemiology and clinical peculiarities of norovirus and rotavirus infection in hospitalized young children with acute Diarrhea in Taiwan, 2009. *J. Microbiol. Immunol. Infect.* 43, 506-514, 2010.
[27] American Academy of Pediatrics: Summaries of infectious diseases. Human Calicivirus infections (Norovirus and Sapovirus). In: Red Book: 2009 Report of the Committee on Infectious Diseases, 28th ed. (Pickering L.K., Baker C.J., Kimberlin D.W., Long S.S., Eds) 2009, 241-242. Elk Grove Village, IL.
[28] Jalilian S., Samarbaf-Zadeh A., Mozhgani S. et al. Relative frequency of norovirus ınfection in children suffering from gastroenteritis and referred to Aboozar Hospital, Ahvaz, Iran. *Jundishapur J. Microbiol*. 5, 355-358, 2012.
[29] Green J., Henshilwood K., Gallimore C.I., Brown D.W., Lees D.N. A nested reverse transcriptase PCR assay for detection of small round-structured viruses in environmentally contaminated molluscan shellfish. *Appl. Environ. Microbiol*. 64, 3, 858-863, 1998.
[30] Wu T.C., Liu H.H., Chen Y.J., Tang R.B., Hwang B.T., Yuan H.C. Comparison of clinical features of childhood norovirus and rotavirus gastroenteritis in Taiwan. *J. Chin. Med. Assoc.* 71, 566-570, 2008.
[31] Victoria M., Carvalho-Costa F.A., Heinemann M.B., Leite J.P., Miagostovich M. Prevalence and molecular epidemiology of noroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro, Brazil, 2004. *Pediatr. Infect. Dis. J.* 26, 602-606, 2007.
[32] Junquera C.G., de Baranda C.S., Mialdea O.G., Serrano E.B., Sánchez-Fauquier A. Prevalence and clinical characteristics of norovirus gastroenteritis among hospitalized children in Spain. *Pediatr. Infect. Dis. J.* 28, 604-607, 2009.
[33] Colomba C., De Grazia S., Giammanco G.M., et al. Viral gastroenteritis in children hospitalized in Sicily, Italy. *Eur. J. Clin. Microbiol. Infect. Dis*. 25, 570-575, 2006.
[34] Narkeviciute I., Tamusauskaite I. Peculiarities of norovirus and rotavirus ınfections in hospitalised young children. *J. Pediatr. Gastroenterol. Nutr.* 46, 289-292, 2008.
[35] Phan T.G., Nguyen T.A., Kuroiwa T., et al. Viral diarrhea in Japanese children: results from a one-year epidemiologic study. *Clin. Lab.* 51, 183-191, 2005.
[36] Rockx B., de Wit M., Vennema H., et al. Natural history of human Calicivirus infection: a prospective cohort study. *Clin. Infect. Dis.* 35, 246-253, 2002.
[37] Pang XL., Joensuu J., Vesikari T. Human calicivirus-associated sporadic gastroenteritis in Finnish children less than two years of age followed pro-

spectively during a rotavirus vaccine trial. *Pediatr. Infect. Dis. J.* 18, 420-426, 1999.
[38] Götz H., Ekdahl K., Lindbäck J., et al. Clinical spectrum and transmission characteristics of infection with norwalk-like virus:findings from a large community outbreak in Sweden. *Clin. Infect. Dis*. 33, 622-628, 2001.
[39] Kirby A., Ashton L. Detection of norovirus infection in the hospital setting using vomit samples. *J. Clin. Virol.* 51, 86-87, 2011.
[40] Chen S.M., Ni Y.H., Chen H.L., Chang M.H. Microbial etiology of acute gastroenteritis in hospitalized children in Taiwan. *J. Formos. Med. Assoc.* 105, 964-970, 2006.