

VIRAL GASTROINTESTINAL SYNDROME IN OUR ENVIRONMENT

A. PATIĆ¹, V. MILOŠEVIĆ¹, I. HRNJAKOVIĆ CVJETKOVIĆ¹, V. PETROVIĆ¹, Z. ŠEGULJEV¹,
S. STEFAN-MIKIĆ², G. KOVAČEVIĆ¹, J. RADOVANOV¹, I. ELEZ¹, A. JOVANOVIĆ GALOVIĆ¹ and
M. ĐILAS¹

¹*Institute of Public Health of Vojvodina, University of Novi Sad, Medical Faculty, 21000 Novi Sad, Serbia*

²*Clinical Center of Vojvodina, Clinic for Infectious Diseases, Medical Faculty, University of Novi Sad, 21000 Novi Sad, Serbia*

Abstract - Viral gastrointestinal syndrome is a cause of morbidity and death worldwide. Infection is spread through contact with an infected person, as well as through contaminated food and water. A lethal outcome is possible in infants and young children due to dehydration and electrolyte imbalance. The study included 141 patients with gastroenteritis from Vojvodina. Real-Time PCR method in stool samples was used to determine the presence of rota-, noro-, and astrovirus nucleic acid. Out of 141 patients with gastroenteritis, 60.3% were confirmed to have one of the three viruses. Rotavirus was significantly more common in children up to 3 years of age (43.3%). Norovirus was more frequently detected in patients older than 20 (50%). These infections started in collectives. Astrovirus was detected in four patients (2.8%). The results confirm the necessity to implement PCR in routine diagnostics for the proper treatment of patients.

Key words: Gastroenteritis, rotavirus, norovirus, astrovirus, Real-Time Polymerase Chain Reaction

INTRODUCTION

Viral gastrointestinal syndrome, i.e. acute gastroenteritis of viral etiology, is still one of the main causes of morbidity around the world, both in developed and developing countries (World Health Organization, 2011; Kuljić-Kapulica et al., 2012; Vuletić et al., 2006; Wilhelmi et al., 2003). According to World Health Organization reports (from June, 2011), viral gastrointestinal syndrome is listed as the fifth leading cause of death worldwide (2.46 million deaths in the world, amounting to 4.3% of total deaths). Likewise, diarrheal syndrome is the second leading cause of mortality in underdeveloped countries (8.2% of total deaths), and the fifth leading cause of death in middle-income countries (4.4% of total deaths). Only in developed countries is gastrointestinal syndrome not among the ten most com-

mon causes of death (World Health Organization, 2011).

A severe clinical picture of the disease can occur in infants and young children. Unless appropriate diagnostic procedures are performed in a timely manner and steps are taken to replenish fluids and electrolytes, viral gastroenteritis can become a cause of mortality, especially in young children (Radlović et al., 1990; Parashar et al., 2003; Bon et al., 1999). Lethality can occur also in the elderly and patients with a weakened immune system (Eckardt et al., 2011; Clark et al., 2004). There are described cases of minor outbreaks of viral gastroenteritis in hospital wards where severely ill patients are hospitalized (Eckardt et al., 2011). Adults can have viral gastroenteritis without pronounced symptoms. They can transmit the infection through the virus excreted

in the stool. The source of the infection in the viral diarrheal disease is human. Infection is transmitted through direct contact via the fecal-oral route, which is also the most important route. However, it is also possible to transfer the infection through contaminated food and water (Kuljić-Kapulica et al., 2012; Eckardt et al., 2011; Clark et al., 2004). The incubation period of the disease is usually from a few hours to two days. The clinical picture is the most severe in children younger than two years of age. The main symptoms are watery diarrhea, abdominal pain, nausea and vomiting. These symptoms can be accompanied by fever, chills, fatigue, muscle pain and headache (Kuljić-Kapulica et al., 2012; Appleton et al., 1975; Lopmam et al., 2003; Levidiotou et al., 2009; Radlović et al., 1990; Holmes et al., 1999).

The most common causes of viral gastrointestinal syndrome are rotaviruses, noroviruses, astroviruses, and even adenoviruses (types 40 and 41). These viruses are relatively resistant in the external environment, which is required for their high contagiousness. Affected individuals excrete large amounts of virus in the stool. Viruses are excreted one day before infection, and 8 to 10 days after infection. In immunocompromised patients, viruses can be excreted for up to 2-3 weeks (Vuletić et al., 2006; Krstić, 2001; Kuljić-Kapulica et al., 2012; Jerant-Patić, 2007).

Many epidemiological studies conducted in developed and developing countries indicate that these viruses represent major etiologic agents of diarrheal diseases. However, the mortality rate is higher in developing countries. Therefore, in order to ensure fast and accurate diagnosis and implement prompt and appropriate treatment of patients, it is necessary to introduce the most advanced diagnostic methods, including molecular diagnostic methods, into the routine work. It is essential to make a prompt diagnosis and distinguish between viral and bacterial enterocolitis, which is especially common in developing countries (Vuletić et al., 2006; Kuljić-Kapulica et al., 2012; Jerant-Patić, 2007).

After a diagnostic exclusion of bacterial causes, fast and precise diagnostic methods (one of the best

among them being the Polymerase Chain Reaction test (PCR test) are required to confirm the viral cause of the diarrheal syndrome. As already mentioned, most often it is caused by rotaviruses (especially in young children), noroviruses (more common in adults in minor local outbreaks within collectives), astroviruses (which are considerably less common in infants and young children, and even more rare in adults). Using differential diagnostics, diarrheal syndrome caused by adenoviruses (types 40 and 41) should be excluded. They usually present a less severe clinical picture and marked respiratory symptoms, and the disease occurs most frequently in children. In children under two years of age, a possible cause of diarrhea are also coronaviruses, which cause sporadic and rare cases of viral diarrheal syndrome, and rarely cause small outbreaks in schools and boarding schools. Diarrhea can be an accompanying symptom, or even a dominant symptom, in enterovirus infections, as well as in hepatitis A and hepatitis E virus infections (in Asia). However, in all these virus infections, apart from diarrhea, there are other dominant symptoms typical for the diseases caused by the mentioned viruses (Jerant-Patić, 2007; World Health Organization, 2011; UNICEF/WHO, 2009; He et al., 2012; Radovanov et al., 2011).

In the temperate climate zone, viral gastroenteritis is somewhat more common during the colder months ("winter diarrhea"), while in the tropics it occurs throughout the year (Vuletić et al., 2006).

The subject of this study is rotaviruses, noroviruses and astroviruses. Rotaviruses belong to the Reoviridae family. They are very resistant in the external environment and are divided into six groups. Group A of rotaviruses is the most common in children with acute gastroenteritis. Group B causes smaller outbreaks of diarrhea in adults, and group C occurs rarely i.e. sporadically, usually in children. Other groups of viruses have been detected only in animals (Vuletić et al., 2006). Noroviruses belong to the Caliciviridae family, with two genera: norovirus (formerly called Norwalk-like), and saporovirus (formerly known as Sapporo-like). Noroviruses are a more frequent cause of diarrheal syndrome than

sapoviruses, and are quite resistant in the external environment. They cause smaller outbreaks in groups and are usually caused and disseminated by food (Hall et al., 2011; Maalouf et al., 2011; Lee et al., 2007; Junguera et al., 2009). Astroviruses belong to the Astroviridae family and are star-shaped. They cannot grow on cell culture, and are therefore less researched. Astroviruses can infect infants and young children, and less frequently adults. Infections occur sporadically. The clinical picture is more severe in children (Herrmann et al., 1991; Kuljić-Kapulica et al., 2012; Jerant-Patić, 2007).

While there is a vaccine for the prevention of rotavirus infections, there is no vaccine against norovirus. Therefore, the prevention of the disease spread is reduced to maintaining sanitary measures of food preparation, and food and water usage. There is also no vaccine against astrovirus (Kuljić-Kapulica et al., 2012).

Diagnostics of viral gastroenteritis can be done using the methods for direct detection of viruses and viral antigens in the stool. Work on cell culture, used exclusively for research purposes and only for rotaviruses, is not applicable for routine diagnostics. Other methods used are electronic and immunoelectronic microscopy, techniques with monoclonal antibodies, RNA electrophoresis in polyacrylamide gel, and molecular PCR techniques. Because of the short duration of the disease, tests of fast and direct identification of pathogens in samples of patient material have a significant advantage over serological diagnostic methods (Jerant-Patić, 2007).

The aim of the study was to determine the frequency and importance of rotaviruses, noroviruses and astroviruses as causative agents of viral gastrointestinal syndrome in different age groups in Vojvodina.

MATERIALS AND METHODS

The study included 141 patients from the territory of Vojvodina, Serbia. Patients were treated for severe gastrointestinal symptoms in the period from Au-

gust 1, 2012 to April 1, 2013. The age of examined individuals ranged from 1 month to 88 years, however, the majority of subjects were children. Most examined patients were hospitalized. Stool samples were brought to the Center for Virology of the Institute of Public Health of Vojvodina, where the molecular diagnostics were performed. Real-Time PCR analysis of samples was used to establish the presence of viral nucleic acid, i.e. to determine the presence of the nucleic acid of rotavirus, astrovirus and norovirus as the most common causes of gastrointestinal syndrome. Viral RNA extraction was performed using the commercial kit QIAamp Viral RNA Mini Kit (Qiagen inc., Valencia CA, USA) according to the manufacturer's instructions. Real-Time PCR was performed using the commercial kit by the manufacturer Sacace (Sacace, Biotechnologies Italy).

The process of reverse transcription was performed under the following conditions: 30 min at 50°C, 15 s at 95°C. The process of amplification included 45 cycles: 10 s at 95°C, 30 s at 60°C, and 10 s at 72°C. Amplification and detection of viral RNA were carried out using the Applied Biosystems 7500.

RESULTS

During an eight-month period, the Real-Time PCR method was used to examine the stool samples of 141 patients from Vojvodina. All patients reported to the doctor with severe symptoms suggestive of acute gastrointestinal syndrome. PCR molecular diagnostics were used to test the samples for rotaviruses, noroviruses and astroviruses as the most common causes of acute diarrheal disease. Out of the 141 tested patients, 85 patients (60.3%) were positive for one of the three viruses. Thus, a viral gastroenteritis caused by one of these three viruses was found in 85 of the patients, of which 75 (88.2%) were hospitalized. This further confirms the severity of the clinical symptoms.

The age of the examined individuals was from 1 month to 88 years. There were 26 patients aged from one month to one year, 14 of whom (53.8%) were positive for one of the three mentioned viruses. There

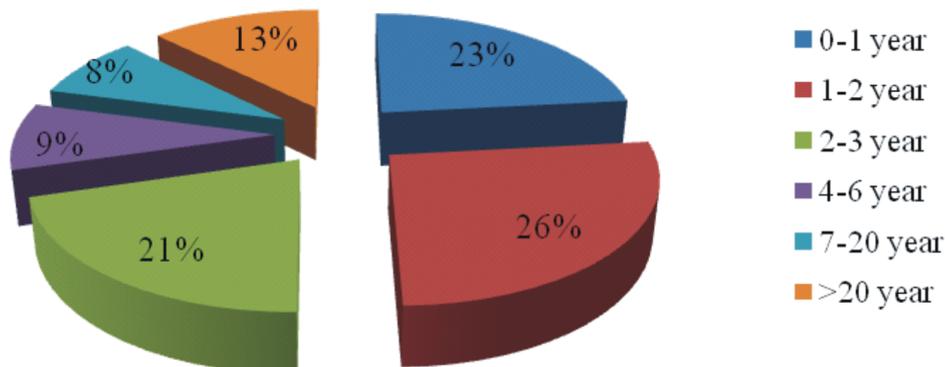


Chart 1. Rotavirus infections by age group

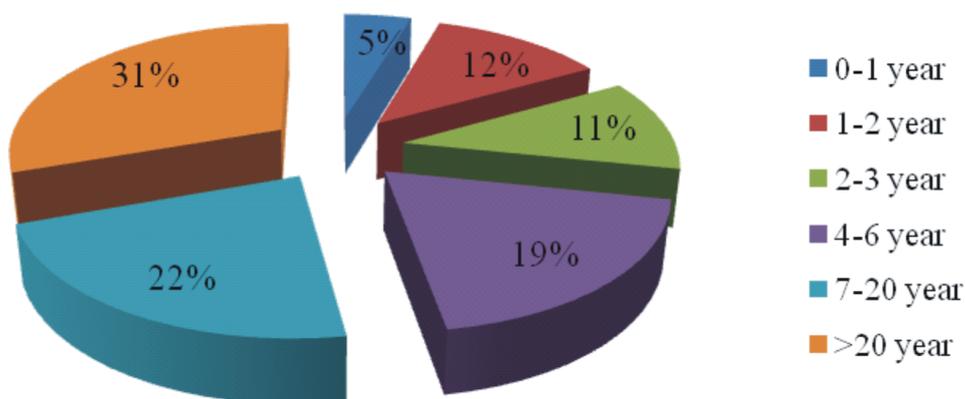


Chart 2. Norovirus infections by age group

were 25 patients aged from 13 months to 2 years, 17 of whom (68%) were positive, and 16 children aged from 25 months to 3 years, 9 of whom (56.2%) were positive for one of the 3 mentioned viruses. In the group of pre-school children (aged 3 to 6 years), 19 patients were examined, 11 of whom (57.9%) were positive, while in the group of those aged 7-20 years, 33 people were examined, 18 of whom (54.5%) were positive. Among the patients older than 20 years of age (aged 20-88 years), stool samples of 22 patients were examined and 16 cases (72.7%) were found to be positive. These data confirm the importance and need for such diagnostic work to be done regularly in patients with acute gastrointestinal syndrome. In the completed questionnaires filled out by the doctors who examined the patients, out of the 85 individuals positive for one of the three tested viruses, the disease started suddenly in 73 patients (85.9%).

Table 1 shows that 42 patients (29.8%) out of the 141 examined patients were positive for rotaviruses. The highest number of confirmed rotavirus infections were found in children aged 13 months to 2 years (48%) and in children in the first year of life (from 1 month to 12 months – 42.3%). In children aged 2 to 3 years, 37.5% were positive for rotaviruses. As can be seen from the results, rotaviruses were statistically significantly the more common cause of acute gastrointestinal syndrome in children under 3 years of age (43.3%), $p < 0.05$. In fact, in pre-school children, these viruses were detected in 15.8% of the patients, in 15.1% of schoolchildren (aged 7 to 20 years), and in 22.7% of patients over the age of 20.

Table 1 shows that noroviruses as causes of acute diarrheal syndrome are the most commonly found in patients older than 20 years of age (in 50% of the

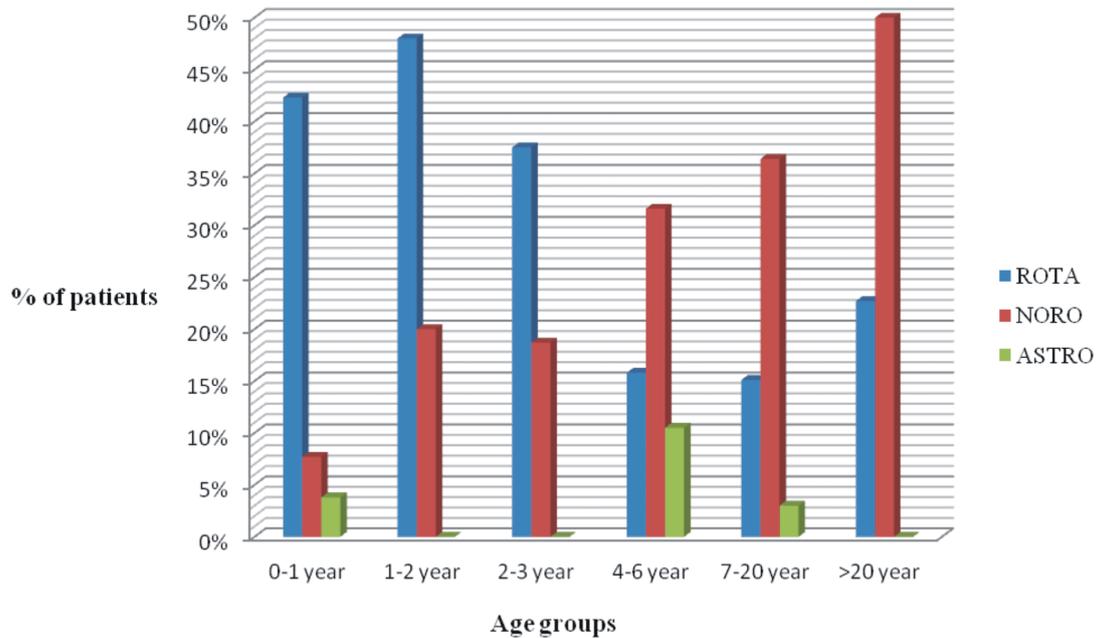


Chart 2. Comparative analysis of patients by age group

examined). This is statistically significantly more frequent compared to other age groups ($p < 0.05$). In the group of pre-school children, the percentage of confirmed norovirus infections was 31.6%; among schoolchildren and adolescents the percentage of acute norovirus infections was 36.4%; this percentage was significantly lower in children younger than three years of age. In the first year of life, the percentage of patients with acute norovirus was 7.7%, in the second year 20%, and in the third year 18.7%. Thus, the PCR test from stool samples of patients with acute gastrointestinal syndrome detected the presence of a norovirus infection in 39 individuals (27.6%) of the 141 examined patients. The data listed in the questionnaire show that these infections were found in small collectives, institutions and families.

The results show that astroviruses were diagnosed individually in patients' stool samples (only in 4 out of the 141 patients, i.e. 2.8%). One patient was under the age of one year, two were of pre-school age, and another case of acute astrovirus infection was detected in a patient of school age.

Finally, according to the results listed in the questionnaire completed by the treating physicians, of the 85 patients who were positive for one of the three viruses tested in the stool samples using the PCR test, in 57 patients (67.1%) the disease lasted up to three days. This supports the necessity of quick diagnostics and an appropriate treatment of patients.

DISCUSSION

The fact that 85 (60.3%) out of the 141 examined patients with signs of acute gastrointestinal syndrome were positive for one of the three tested viruses is in agreement with the results of other authors, and confirms the need to regularly perform virological diagnostics in these patients, using quick and accurate diagnostic tests such as molecular methods (He et al., 2012; Pativada et al., 2012; Soriano-Gabarró et al., 2006; Rovida et al., 2013). Timely and appropriate virological diagnostics enabled the prompt and efficient treatment of patients, as evidenced by the fact that the disease lasted from 1 to 3 days in 67.1% of patients. This is stated in the WHO recommenda-

Table 1. Rota-, noro- and astrovirus gastroenteritis by patients' age

Age	ROTA positive/tested (% positive)	NORO positive/tested (% positive)	ASTRO positive/tested (% positive)	Total
0-1 years	11/26 (42.3%)	2/26 (7.7%)	1/26 (3.8%)	14/26 (53.8%)
1-2 years	12/25, (48%)	5/25 (20%)	0/25 (0)	17/25 (68%)
2-3 years	6/16 (37.5%)	3/16 (18.7%)	0/16 (0)	9/16 (56.2%)
4-6 years	3/19 (15.8%)	6/19 (31.6%)	2/19 (10.5%)	11/19 (57.9%)
7-20 years	5/33 (15.1%)	12/33 (36.4%)	1/33 (3%)	18/33 (54.5%)
> 20 years	5/22 (22.7%)	11/22 (50%)	0/22 (0)	16/22 (72.7%)
Total	42/141 (29.8%)	39/141 (27.6%)	4/141 (2.8%)	85/141 (60.3%)

tions (UNICEF/WHO, 2009).

Rotavirus infections demonstrated in 29.8% of the patients were statistically significantly more frequent in children under 3 years of age (43.3%). In addition, they were usually diagnosed in children in the second year of life (48%), which is also consistent with the results of a large number of authors from various countries (Parashar et al., 2003; Lopmam et al., 2003; UNICEF/WHO, 2009).

Norovirus infections, demonstrated in 27.6% of the examined patients, were significantly more common in adults (older than 20 years of age; diagnosed in 50% of patients). The next age group according to the prevalence of norovirus infections were school-children and adolescents (at 36.4%), as well as pre-school children (at 31.6%). The lowest number of norovirus infections was found in children younger than 3 years of age, especially in children under one year of age (7.7%). The resulting percentages of frequency of norovirus infections, data on their distribution according to the age of the patients, and the fact that these infections were observed in smaller collectives, institutions and families, is in line with most findings from literature (UNICEF/WHO, 2009; Glass et al., 2009; Liu et al., 2010).

The fact that astroviruses were diagnosed significantly less frequently than rotaviruses and noroviruses (only in four cases – 2.8%), in children, also agrees with the findings from literature (UNICEF/WHO, 2009; Herrmann et al., 1991; Bon et al., 1999).

CONCLUSION

This research confirms the importance of rotaviruses and noroviruses as causal agents of viral gastrointestinal syndrome in different age groups in Vojvodina. At the same time, this work reaffirms the need for virological diagnostics to be undertaken regularly in patients with acute diarrheal syndrome, using rapid and sensitive diagnostic tests such as molecular methods (PCR techniques). It was confirmed that a rapid and accurate diagnosis allows prompt and appropriate treatment of patients (particularly young children), which shortens the duration of the disease. This undoubtedly minimizes the potential complications of the disease.

Rotavirus infections were more common in young children (under 3 years of age), and most common in children in the second year of life. Norovirus infections were more frequently found as small outbreaks within collectives, especially in individuals

older than 20 years of age, followed by schoolchildren. Astrovirus infections in this study were found only in individual cases in children.

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