

The Diagnosis of Norovirus Infections in Hospitalized Children and Adolescents with Acute Gastroenteritis: a Study from Pilsen, Czech Republic

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Summary

Background: Norovirus-associated sporadic gastroenteritis is an important cause of illness in Western Europe. However, at present, little information on the role of norovirus in sporadic gastroenteritis in Central Europe is available. Our study aimed at providing an assessment of their significance in hospitalized children and adolescents with acute gastroenteritis using ELISA test at the time of their introduction.

Methods: A prospective hospital based study of the etiology of acute gastroenteritis was undertaken in a total of 618 patients (mean age 39.8 months, range 0-228), who were hospitalized at the Charles University Hospital in Pilsen. All subjects were monitored in six fragmented periods during the years 2003 and 2004, 2006 and 2007. Clinical and laboratory data were processed, norovirus antigens in stools were detected using the EIA kits IDEIA Norovirus, DakoCytomation.

Results: A norovirus infection was confirmed in 62 cases, i.e. 10.0 % of all patients with acute gastroenteritis. Vomiting was the most common symptom, recorded in 95.2 % of all the patients with norovirus infection. No severe extragastrointestinal complications were detected. The average interval between initial symptoms and the beginning of hospitalization was considerably shorter in patients with norovirus infection (1.3 days) compared with patients with rotavirus infections (2.4 days). The frequency of *Salmonella* spp., rotavirus, *Campylobacter* spp. and enteric adenovirus was 15.4 %, 11.2 %, 3.9 %, 3.6 %, respectively.

Conclusions: Our findings confirm the clinical importance of noroviruses as a causative agent of acute gastroenteritis in children and teenagers in the region of a Central European country. Identification of norovirus infection should be included in the routine screenings of sporadic cases of acute gastroenteritis.

Key words: ELISA, etiology, norovirus, gastroenteritis

Souhrn

Pazdiora P., Táborská J., Švecová M., Sýkora J.: Diagnostika novovirových infekcí u hospitalizovaných dětí a mladistvých s akutní gastroenteritidou. Plzeňská studie

Norovirové sporadické gastroenteritidy jsou důležitou a významnou nemocí v západní Evropě. V současnosti je ale k dispozici pouze málo informací o jejich roli v etiologii sporadických gastroenteritid ve střední Evropě. Naše studie se zaměřila na hodnocení jejich významu v etiologii akutních gastroenteritid u hospitalizovaných dětí a adolescentů za použití testů ELISA v době jejich zavádění.

Metody: Prospektivní studie etiologie akutních gastroenteritid byla provedena u 618 pacientů (průměrný věk 39,8 měsíců, rozmezí 0-228), kteří byli hospitalizováni ve Fakultní nemocnici v Plzni. Pacienti byli sledováni v 6 oddělených obdobích během let 2003 a 2004, 2006 a 2007. Byla zpracována jejich klinická a laboratorní data, k detekci novovirových antigenů ve stolici byly používány kity ELISA (IDEIA Norovirus, DakoCytomation).

Výsledky: Norovirová infekce byla potvrzena u 62 osob, tj. u 10,0 % pacientů s akutní gastroenteritidou. Nejčastějším příznakem bylo zvracení, které bylo zaznamenáno u 95,2 % pacientů s norovirovou infekcí. Nebyly zaznamenány žádné závažné extraintestinální komplikace. Průměrný interval mezi prvními příznaky a začátkem hospitalizace byl podstatně kratší u pacientů s norovirovou infekcí (1,3 dne) v porovnání s pacienty s rotavirovou infekcí

(2,4 dne). *Salmonella* spp., rotaviry, *Campylobacter* spp. a střevní adenoviry se v etiologii uplatnily v 15,4 %, 11,2 %, 3,9 % a 3,6 %.

Závěry: Naše nálezy potvrzují klinický význam norovirů v etiologii akutních gastroenteritid u dětí a adolescentů v regionu středoevropské země. Identifikace norovirových infekcí by měla být zahrnuta do rutinního screeningu u sporadických akutních gastroenteritid.

Klíčová slova: ELISA – etiologie – noroviry – gastroenteritida.

Introduction

Acute gastroenteritis continues to be an enormous problem both in developing and in developed countries. Nevertheless, a pathogen is currently identified in only a small proportion of cases. During the past decades, a dramatic increase in the number of newly recognized etiological agents of gastroenteritis has been identified. Norwalk agent and Norwalk-like viruses were the first viruses connected with gastroenteritis at the beginning of the 1970s. However, the interest in their diagnosis has only recently increased because of the increase in epidemics and nosocomial infections caused by these viruses, classified according to current taxonomy as noroviruses [3]. Noroviruses are genetically highly variable with a single-stranded RNA genome. They are the major cause of epidemic non-bacterial gastroenteritis around the worldwide. Additionally, noroviruses also are frequently involved in sporadic cases of gastroenteritis. Diseases caused by noroviruses are self-limited in most cases. The disease is restricted to a few days [5,6,12,13,17,27,28]. Due to this significance more accessible methods (EIA, immunochromatography, PCR) than electron or immunoelectron microscopy have began to be applied in clarifying their importance [2,7,14,22,24,26].

Only a limited number of studies have addressed this issue in European countries to date, and moreover, there is very limited data about their occurrence in the etiology of sporadic gastroenteritis in Central Europe [2,25,29].

Thus, on the basis of the possibility of testing by means of the commercially available ELISA kit, we aimed at providing the assessment of their significance in hospitalized children and teenagers with acute gastroenteritis at the time of their introduction in the Czech Republic.

Material and Methods

A prospective pilot hospital based study was undertaken at two departments of the Charles University Hospital (Pilsen, Czech Republic) in six fragmented periods between 2003 and 2004, 2006 and 2007, i.e. October to November 2003 and March to April 2004, July to August 2006, October to

November 2006, January to March 2007, and April to July 2007. The diagnosis of norovirus infections in children and teenagers, hospitalized at the Clinic of Infectious Diseases and the Department of Paediatrics with acute gastroenteritis, was performed as a part of the determination of the etiology of gastrointestinal infections at the time of initial evaluation. An examination of native stools was undertaken by the laboratory of virology at the Institute of Microbiology of the Charles University Hospital in Pilsen. For the detection of antigens the IDEIA™ Norovirus K6043 and K6044 (DakoCytomation Ltd.) kits were used between 2003 and 2004, 2006 and 2007 respectively according to the manufacturer's instructions. K6043 detects both genogroups I and II and distinguishes between them, kit K6044 does not distinguish between them.

Clinical and laboratory data were collected from the patients medical charts. All data were fed into the computer database EPI-Info 6.04. Descriptive data and the results were processed statistically using chi-square test. The level of significance was set at 0.05.

Results

A total of 618 patients of both sexes (326 males, 292 females), the mean age of the children was 39.8 months (range 0-228 months) were considered eligible during the study period. All subjects were admitted for their first specialized professional assessment of acute gastroenteritis and included in this prospective study. In the first study period of our project 63 patients were examined at the time of admission. A noroviral infection was confirmed in 4 of 63 cases (6.3 %). In the second period of this study a laboratory measurement was performed in 90 patients at the time of admission, and in 2 of 90 patients (2.2 %) a norovirus infection was confirmed. All confirmed norovirus infections belonged to genogroup II. In the other four periods norovirus infection was confirmed in 11, 11, 19, 15 cases (11.8 %, 11.8 %, 20.4 %, and 8.1 % respectively). An infection was confirmed in 62 of 618 subjects at the time of initial evaluation, i. e. 10.0 % of all patients. In 8 of them mixed infections with other viral or bacterial agents norovirus infections were seen. The frequency of norovirus infection was not significantly different ($\chi^2 = 0.60$, $P=0.44$) among children ≥ 5 years (17/145) and children <5 years (45/473). In colder months of the year (from October to March) 36 out of the 413 examined patients norovirus infections were confirmed, in other months (from April to September) the

Table 1. Basic characteristics of 62 patients with norovirus infection (Pilsen 2003/2004,2006/2007)

| Parameter | Values |
|---|---|
| Symptoms before hospitalization: vomiting diarrhoea fever | 59 children, 43 children, 23 children |
| Time of hospitalization (days) - average (min-max) | 2.6 (1-6) |
| Duration of diarrhea (days) - average (min-max) | 1.9 (1-4) |
| Number of stools per day - average (min-max) | 5.2 (1-13) |
| Duration of vomiting (days) - average (min-max) | 1.0 (0.3-2.0) |
| Number of vomiting episodes per day - average (min-max) | 2.1 (1-7) |
| Dehydration 0.1-5.9 % 6.0-9.9 % ≥10.0 % | 42 children 18 children - |
| Fever (>37.5 °C) | 41 children (37.6-39.5) |
| Duration of fever (days) - average (min-max) | 1.2 (1-3) |
| Duration of infusion therapy (hours) - average (min-max) | 22.5 (9-48) |

Table 2. Biochemical and haematological examination of patients with norovirus infections (Pilsen, 2003/2004, 2006/2007)

| Test | Unit | Values Normal | Reduced | Increased |
|------------|--------|---------------|---------|-----------|
| CRP | mg/l | 1-10 | - | 15 |
| Na | mmol/l | 132-145 | 8 | - |
| K | mmol/l | 3.6-5.2 | 2 | 2 |
| Urea | mmol/l | 1.4-6.7 | - | 25 |
| Glucose | mmol/l | 1.7-5.3 | - | 27 |
| Creatinine | μmol/l | 16-72 | - | 26 |
| AST | μkat/l | <0.67 | - | 22 |
| ALT | μkat/l | <0.67 | - | 10 |

Table 3. Etiology in patients examined for norovirus infection (Pilsen, 2003/2004, 2006/2007)

| Age | Number of examined | Etiology | | | | | | |
|--|--------------------|--------------|---------------|-----------------|--------------|-----------|--------|---------|
| | | Rota-viruses | Adeno-viruses | Norovi-ruses(%) | Salmonel-las | C. jejuni | Other | Mixed* |
| 0-6 months | 142 | 10 | 2 | 2(1.4) | 3 | 1 | | 2 |
| 7-12 months | 64 | 12 | 2 | 6(9.4) | 7 | 1 | | 3** |
| 13-24 months | 115 | 17 | 6 | 12(10.4) | 22 | 5 | 1 | 2* |
| 25-36 months | 65 | 7 | 3 | 9(13.8) | 11 | 3 | 1 | 2* |
| 37-48 months | 52 | 1 | 3 | 7(13.5) | 14 | 1 | 1 | 1* |
| 49-60 months | 35 | 5 | 2 | 4(11.4) | 5 | 2 | | |
| 5-9 years | 104 | 12 | 4 | 9(8.7) | 28 | 7 | 2 | 2** |
| 10-14 years | 30 | 2 | | 4(13.3) | 5 | 4 | 1 | |
| 15-19 years | 11 | 3 | | 1(9.1) | | | | 1* |
| Total (%) | 618 | 69(11.2) | 22(3.6) | 54(8.7) | 95(15.4) | 24(3.9) | 6(1.0) | 13(2.1) |
| Interval between the beginning of the disease and average hospitalization (days) | | 2.4 | 2.9 | 1.3 | 2.1 | 2.1 | 1.0 | . |
| Average hospitalization (days) | | 3.2 | 3.1 | 2.6 | 4.0 | 3.2 | 5.2 | . |

* 8 mixed norovirus infection

number was 26 (205) infections ($\chi^2 = 2.39$, $P=0.12$).

Eighteen patients (29.0 %) out of the 62 presented the occurrence of gastrointestinal disease within the household before the onset of their disease (parents, brothers and sisters, grandparents, other relatives). Before admission to the hospital, 59 patients had suffered from vomiting, 43 from diarrhoea and 23 had a fever. During hospitalization, 50 patients suffered from diarrhoea (all of them with absence of blood or mucus in their stools), 23 vomited and 41 had a fever. The general characteristics of the whole sample are listed in Tables 1 and 2 summarizing the basic clinical and laboratory characteristics. There were no signs of extragastrointestinal symptoms such as severe dehydration and peripheral collapse. As shown in Table 3 the average time of the hospitalization was 2.6 days (range 1-6). In the clarified etiology in examined patients the most frequent agents was *Salmonella spp.* as can be seen in Table 3.

Discussion

This pilot study investigated the occurrence of norovirus infections in acute gastroenteritis in the pediatric population who resided within the same geographic area. In this analysis performed in the six periods, we confirmed that norovirus infections in our region also play a significant role in the etiology of gastrointestinal diseases in children and teenagers. Our results suggest that together with rotavirus, astrovirus, and enteric adenovirus infections they are the most common viral causes of sporadic gastroenteritis [1,6,11,16].

On the other hand, our study demonstrated that compared with above mentioned rotavirus and adenovirus infections the hospitalization of patients with norovirus infections is shorter. Our study clearly presented that the infection lasts a short time and is mostly without severe extragastrointestinal complications. It was also confirmed, however, that in our conditions the infection probably occurs all the year round without the seasonal peak in the colder months. Polish authors recorded the seasonal peak between September and December [29]. Surprisingly, these data are not consistent with findings of other authors [17,18,20]. In this regard, we count our data as preliminary; for the objective evaluation a long continuous observation is necessary. When compared with other etiological agents, it turned out that a rapid, dramatic occurrence of symptoms,

particularly vomiting, can lead to early admittance to hospital compared with other infectious agents [15,23]. Moreover, a number of epidemiological and clinical characteristics are similar to rotavirus infections [30]. According to our results the occurrence of norovirus infections among inpatients is similar in all age groups studied. A lower interception of noroviruses in the first two periods could have been influenced not only by the stated lower sensitivity of the K6043 kit, but also by a possible lower occurrence of noroviruses in the population.

There is now strong evidence that noroviruses are an important cause of sporadic gastroenteritis in countries such as Spain, Italy, the United Kingdom and Ireland [4-6,10,12,16,19,31], but there is still little information on this subject in Central Europe [2,25,29]. Our study investigated for the first time the epidemiology of norovirus infection among Czech children regarding sporadic acute gastroenteritis in more seasons, with the use of ELISA tests. We described a similar rate of occurrence to other European studies. If they were included in the routine diagnostics of gastroenteritis in hospitalized patients, there would be a desirable increase in the percentage of etiologically confirmed diseases, which would provide physicians with more valuable data and prevent the unnecessary use of antibiotics. In the light of our findings, and in line with others, we conclude that further exploration including large patient numbers and different geographical regions is needed. The introduction of more sensitive RT-PCR would also permit the monitoring of individual genotype circulation, which is already being performed in certain countries [4,13,16,19,25,31].

After a dramatic development of clinical symptoms (vomiting, febrile diarrhoea), a quick recovery can be observed within several days as shown in our study. A high degree of contagiousness is typical of the infection. Fretz et al described an occurrence of a previous gastrointestinal infection in contacts, mostly with family members, in 39% of the patients with confirmed sporadic noroviral infection [10]. A significant proportion of the transmission via vomit also tends to be detected in epidemic patients [9]. In the context of our results, these data are also consistent with our findings concerning the presence of the disease in family members of 29.0 % of our patients, and even in our conditions, frequent family mini-epidemics is very likely to develop.

In our study, we used the ELISA kit by Dako for diagnosis of norovirus infection. The sensitivity of this kit has been compared to products by other

manufacturers, as well as to other methods. The sensitivity, when compared with EM and RT-PCR, was 24-66 %, and the specificity was 85-99 % [8,26]. At present, novel kit products (K6044) are available providing higher sensitivity and specificity useful in clinical practice. These kits, which have a positive predictive value in relation to PCR according to the data of the manufacturer, have the same specificity and a negative predictive value in relation to PCR.

In conclusion, considering all the above, we conclude, that sensitive ELISA kits should be recommended for basic assessment of the occurrence of sporadic, and epidemic gastroenteritis regarding norovirus infection.

The study was supported by a financial grant from the Ministry of Education, the Czech Republic (VZ MSM 0021620819)

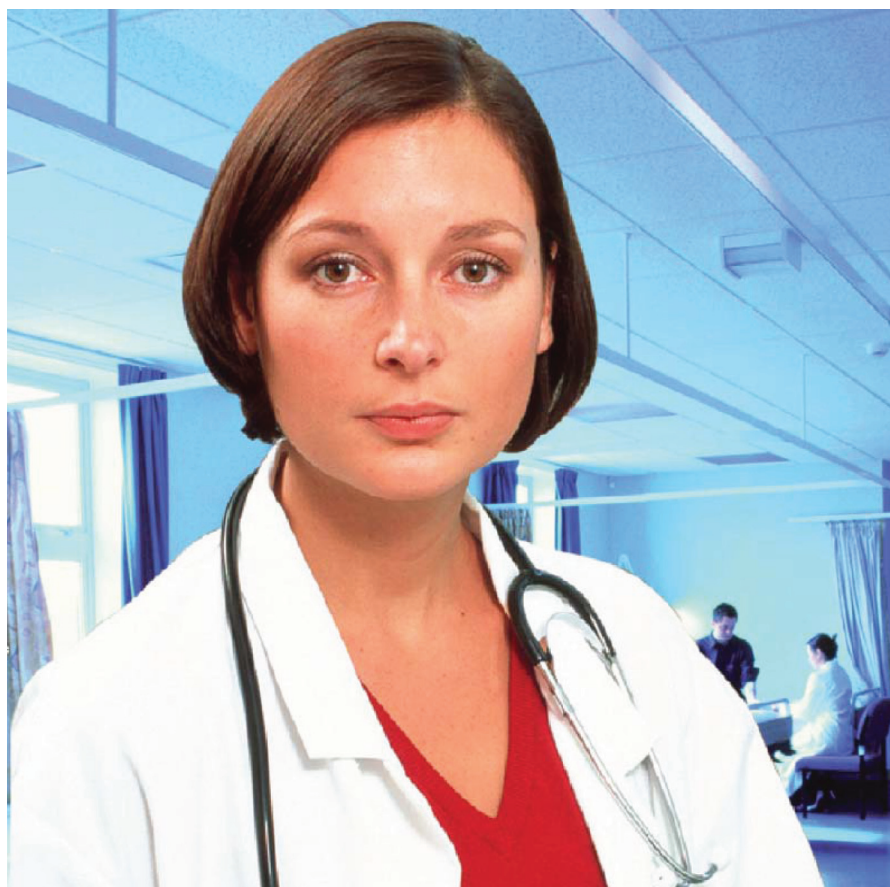
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Do redakce došlo 23.7.2009

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Norovirus ELISA

- Noroviry - nejčastější příčina virových gastroenteritid
- Rychlý průkaz antigenů -
- možnost omezení šíření epidemie
- Výsledky testu do 2 hodin
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