

NOROVIRUSES - A HIDDEN THREAT

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(Mini review)

ABSTRACT

Acute nonbacterial gastroenteritis is ubiquitous, and noroviruses are considered to be among the most common etiological agents. Noroviruses affect people of all ages. As a rule, infections caused by them are mild and self-limiting within 1-3 days but there is always a risk of a more severe course, especially in infants and the elderly. Being considered as mild and fast transient, noroviruses receive less attention than other infectious pathogens. The clinical and economic burden of norovirus gastroenteritis is often underestimated. Detection of the etiological role of noroviruses is essential not only from the clinical point of view, but also from the economic one because of the damage that norovirus infections cause to tourism and food industry. At present, prevention and counter disinfection measures are the only weapon against norovirus infections.

In this narrative review, results from a non-systematic search on the recent literature on noroviruses are presented. The review describes the basic biological characteristics of noroviruses, their genetic diversity and current classification, as well as the epidemiological aspects of the norovirus infection, its clinical manifestation, the diagnostic approaches, prevention and control measures and current state-of-the art for norovirus vaccine development.

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Keywords: *noroviruses, acute gastroenteritis, diarrhea, vomiting*

INTRODUCTION

Diarrheal diseases are among top 10 causes of death (1). Acute nonbacterial gastroenteritis is the second most important infection of all infectious diseases, and human noroviruses (HuNoVs), especially after the introduction of the rotavirus vaccine, are now arguably considered the leading etiological factor among all age groups (2). Unlike rotaviruses, which are the leading causes of acute intestinal disorders in children under 5 years of age, HuNoVs attack people of all ages. However, following the introduction of the rotavirus vaccine in some countries, HuNoVs tend to be the dominant etiologic agent in pediatric cases of acute gastroenteritis (3).

It has been found that one in every five cases of acute nonbacterial infectious gastroenteritis is caused by HuNoVs, which accounts for almost 700 million clinically manifested infections per year, one third of which are in children under 5 years of age (4). And although mortality, especially among children, is observed mainly in developing countries, HuNoVs are the same problem for both these countries and the countries with the highest gross domestic product per capita. Globally, norovirus infections cost humanity more than \$ 60 billion a year: \$ 4.2 billion in direct health care costs (including hospitalizations) and \$ 56.2 billion in lost economic benefits - lost working and school days, emergency disinfection, quarantine measures, etc. (5).

Despite these startling numbers, HuNoVs remain relatively unknown compared to other infectious pathogens. They are almost ignored by the media, do not receive attention from politicians, and programs that systematically fund research on them, are almost non-existent.

BASIC BIOLOGICAL CHARACTERISTICS

Virions of HuNoVs are small (approximately 27 nm in diameter), non-enveloped with

icosahedral geometry, and the virus particle morphology is spherical without any protruding spikes. The capsid is composed of 90 dimers of viral protein VP1 arranged in such a way that cup-like hollows are seen under electron microscopy (6). The capsid encloses the virus genome which consists of a relatively small, single-stranded, non-segmented, positive-sense, linear RNA, approximately 7500 nucleotides in length. The genome is organized into three overlapping open reading frames (ORF1–ORF3). ORF1 encodes a large polyprotein which is further cleaved into six smaller non-structural proteins (NS1/2 to NS7) by the virus specific protease (NS6). ORF2 and ORF3 encode for the major (VP1) and minor (VP2) viral capsid proteins, respectively. The 5' end of the genome is covalently linked to VPg, and the 3' end contains a polyadenylated tail (7).

RESISTANCE IN THE EXTERNAL ENVIRONMENT

HuNoVs are extremely resistant in the environment. Outside the human host they remain viable for weeks and even months on surfaces and in water (8). The lack of a lipid envelope makes norovirus virions resistant to organic solvents such as ethanol, ether and chloroform. Capsids remain stable at neutral and acidic pH and remain intact at temperature up to 55°C (9). These characteristics of HuNoVs greatly contribute to the high transmission rate of the infection, especially in closed groups and premises.

CLASSIFICATION AND GENETIC DIVERSITY

HuNoVs belong to the *Norovirus* genus in the family *Caliciviridae*. The genus is currently subdivided into 7 genogroups based on the complete nucleotide sequence encoding the major capsid protein VP1 (10). Genogroups are denoted by the capital Latin letter G and the corresponding Roman numeral. Viruses from genogroups I, II and IV (GI, GII, GIV) are human pathogens, three genotypes of GII are detected in feces from swine and one genotype from GIV is detected in cats and dogs. The rest genogroups

include viruses that infect only animals and humans are not affected by them. Genogroups are further divided into genotypes denoted by an Arabic numeral, and genotypes are subdivided into variants. In general, genogroups differ by about 40–60 percent of their amino acid sequence and genotypes by about 20–40 percent (11). Since the mid-1990s, GII.4 has been the most prevalent genotype worldwide and it is responsible for 70–80% of all norovirus-associated gastroenteritis outbreaks worldwide (12). Chhabra et al., 2019, propose an update of the classification of noroviruses and have expanded the number of genogroups to 10 (13). The abundance of a variety of different norovirus strains is due to their extremely high variability. There is a continuous process of genetic and antigenic diversification and a new variant is generated every 2-3 years due to the accumulation of point mutations or recombination events. Thus, in the winter of 2014/2015, the new variant GII.17 Kawasaki emerged in Japan, which quickly replaced the dominant GII.4 Sidney (14). In the same season, the new variant was detected in Europe, in Italy (15), and in the summer of 2015 it was already established in Bulgaria (16). The GII.17 genotype has become the predominant strain in some parts of Asia (17).

EPIDEMIOLOGICAL ASPECTS OF NOROVIRUS INFECTION

In the absence of a suitable and accessible laboratory model for studying HuNoVs and the infection caused by them, their epidemiology has been elucidated thanks to healthy volunteers experimentally infected with HuNoVs. Thus in fact, the virus itself was discovered, the fecal-oral mechanism of transmission and the duration of virus shedding in the environment were proven (18).

Data from human challenge studies have shown that the virus is found in the feces of the infected shortly before the illness, and is present for up to several weeks after patient's full recovery.

There may be considerable heterogeneity in the number of viruses that are shed (19). However, the titers of shed virus are the highest during the clinical manifestation and a few days after. Vomiting appears to facilitate transmission due to the formation of fine infectious aerosols during fountain vomiting. It has been found that about 30 million virus particles are released in a single vomiting session. (20). Virus deposited on surfaces can also cause outbreaks (21) and virus can be transferred by hand contact followed by ingestion (22).

HuNoVs are highly contagious (23). Routes of transmission (food-borne, water-borne and contact-to contact) facilitate transmission within closed or narrow spaces. That is the reason norovirus infection to be often referred as 'the cruise disease' (24). In the enclosed space of a cruise ship, along with the presence of a significant number of people, literally anyone can be infected in a short period of time. However, the same rule also applies to closed and semi-closed institutions such as boarding houses, hospices, places of imprisonment, etc.

Different genotypes and variants show relatively different "behaviors" regarding mode of transmission, characteristics of the outbreak, and clinical picture (25). For example, GII.4 variants are more often associated with vomiting and prolonged diarrhea, and with a higher number of diarrheal bowel movements. GII.4 is much more often associated with transmission via the fecal-oral mechanism from direct human-to-human contact than with other known factors of transmission (contaminated food, water, surfaces).

Norovirus genotypes other than GII.4, such as GI.3, GI.6, GI.7, GII.3, GII.6 and GII.12, are more commonly transmitted through contaminated food. It has also been found that GI strains cause waterborne infections much more often than GII strains (26). Foodborne route of transmission is very important for the global spread of HuNoVs (27). This can happen when food is prepared by an infected staff member or at even earlier

stages - when growing, transporting or storing food. Numerous food outbreaks, as well as sporadic cases, are caused by viruses that contaminate products that are usually consumed fresh or with minimal processing such as leafy vegetables, strawberries, raspberries, sun-dried tomatoes, and seafood. Cases of norovirus infections due to oyster consumption have been reported in many countries (28,29,30). Such outbreaks may simultaneously involve more than one norovirus strain, which in turn can lead to viral recombination events (26). Shellfish can be infected when grown on farms contaminated with wastewater, as well as ground fruits watered with contaminated water (31).

With regard to the risks of norovirus contamination during irrigation, it has been found that, on the one hand, possible fecal contamination in irrigation facilities can lead to virus "sticking" to the surface of fruits and vegetables, and on the other hand, an infectious virus can reach the fruit, entering through the root system of the plant and spreading by its vascular tissue (32).

CLINICAL MANIFESTATION AND COMPLICATIONS

In healthy adults, HuNoVs cause a self-limiting disease lasting one to three days, but in the elderly, young children and immunocompromised patients, these viruses can cause a long-lasting infection (33,34,35). Clinical course is usually characterized by a sudden onset of nausea and several debilitating and almost fountain vomiting, quickly followed by repeated diarrhea without pathological impurities. In children, vomiting is a more common symptom than diarrhea, while in adults, it is the opposite – diarrhea is the leading symptom. Body temperature may be slightly elevated. There are complaints of abdominal cramps, malaise, severe fatigue. As both vomiting and diarrhea are profuse, patients quickly lose a relatively large amount of fluid and dehydration-related complaints come to the fore. The patient feels so bad that he is unable

visit the doctor. However, the complaints are short-lived, severe clinical manifestation lasts no longer than a day, rarely two days. Average duration of the disease is 12-60 hours. A very quick recovery comes and according to the patient, the need to visit a doctor disappears. That is the main reason for not knowing the real frequency and burden of disease, as well as the economic impact, especially of sporadic norovirus infections. Despite rapid and complete recovery, however, shedding of viable HuNoVs through the feces continues for several days and even up to two weeks imposing a real threat to an epidemic outbreak.

No matter how mild the course of a norovirus infection is, it poses dangers especially for the vulnerable age groups - infants, young children, and the elderly. HuNoVs are an important risk factor for complications and increased mortality in immunocompromised individuals, including transplant patients and those receiving immunosuppressive therapy (36). People suffering chronic diseases, especially of the gastrointestinal tract, are also at a relative risk. In young children, body temperature can be greatly elevated, the general condition can deteriorate very quickly and lead to febrile seizures (37,38). Cases of necrotizing enterocolitis in children have also been reported (39). In adult patients, on the other hand, underlying inflammatory bowel disease may worsen (40). There is evidence that severity of the disease is increased if the patient takes statins to regulate cholesterol levels (41). Extraintestinal manifestations of norovirus infections are considered a rare phenomenon, and their mechanisms are still unclear. Upon a detailed review of the available literature, Ho et al. (2020) found rare reports of norovirus-induced hepatitis (42).

DIAGNOSTICS

Noroviruses were discovered in 1972 by electron microscope analysis of stool samples from an outbreak of acute non-bacterial gastroenteritis with unknown aetiology in an elementary school

in Norwalk, Ohio, USA (18). Electron microscopy is a relatively insensitive method with a detection limit of approximately 10^6 norovirus particles per gram of feces and it requires skilled personnel and sophisticated equipment (43).

At present, reverse transcriptase polymerase chain reaction (RT-PCR) is the most frequently used technique for detection of norovirus RNA in clinical specimens (feces or vomitus), contaminated food, water, or fomites, and it is considered as "the gold standard" in norovirus diagnostics. Due to the high sequence variability among norovirus strains, most RT-PCR assays use primers that target a conserved region in ORF1 (coding for the viral RNA-dependent RNA-polymerase) or a conserved region in the ORF1-ORF2 junction region (44). While RT-PCR assays provide only qualitative results, real-time quantitative qRT-PCR assays allow rapid detection as well as comparison of viral RNA levels. A major concern regarding the PCR techniques is the inability to discriminate between inactivated and potentially infectious virus particles (45). Infectious viruses can be detected by cell-culture based techniques but in the case for HuNoVs these are available for limited laboratories (46). Immunochromatographic and enzyme immunoassays (ELISA) are also available to detect norovirus antigen in stool samples. The advantage of the latter assays compared to RT-PCR based methods, is the simplicity and rapidity of the assay. No specialized equipment is required and results can be ready within few hours.

The sensitivity of rapid immunochromatographic tests varies considerably and they may be useful for the early diagnosis of outbreaks, for which a greater number of stool samples is tested and only a few positive results are enough for etiologic confirmation. However negative samples cannot be considered true negatives and must be controlled by RT-PCR (47).

Comparative studies with commercial ELISA assays show a wide range of sensitivity and specificity values (48). When ELISA is used,

the probability of detecting positive samples is lower if small numbers of samples are tested. Using ELISA instead of RT-PCR for the detection of norovirus in stool samples will result in a considerable number of false negative outbreaks unless a minimum of 6 samples per outbreak are tested. This precludes use of ELISA assays for individual patients, but diagnosis of outbreaks may be possible (49). Nevertheless, due to the high genetic and antigenic diversity of norovirus strains, certain genotypes can be missed with these assays and therefore they should preferably be used in combination with a confirmation of negative samples by RT-PCR (50).

PREVENTION AND TREATMENT

Outbreak management focuses on preventing further spread of the virus by containment of infected individuals and hygienic measures. Hand washing is the key hygienic action and has demonstrated to prevent further spread of health-care associated infections (51). Because of their extreme stability in the environment, HuNoVs require chemical disinfection with high concentration of hypochlorite, detergents based on hydrogen peroxide or phenolic-based cleaning solutions (52).

Historically, the development of a norovirus vaccine has been hampered by the lack of a small-animal model and a cell culture system, both of which have been described only recently, and licensed vaccines are not yet commercially available. Nevertheless, there are currently three types of norovirus vaccines in different phases of testing – non-replicating virus-like particles (VLPs), P particles, and recombinant adenoviruses. All these vaccine platforms have challenges and limitations but the real problem is which genotypes should be included in the vaccine. A vaccine including the prototype GI.1 strain has been developed but its low cross reactivity with the dominant GII.4 genotype imposes rather a combined approach (53). Some norovirus vaccines have now completed Phase I and Phase II clinical trials (54).

There are several types of experimental antiviral drugs that are in the process of their very early development. The only antinorovirus agent that has almost completed a clinical phase of testing is nitazoxanide (NTZ) (55). There is controversial evidence for the efficacy of NTZ, some studies prove its effectiveness, others reject it. However, NTZ is one of the therapeutic options (excluding ribavirin, immunoglobulins and maintenance care) for patients with persistent infections (56).

CONCLUSION

Each discovery is followed by a series of new questions: what is the role of the human microbiome in norovirus infection, what is the role of the immune system or the blood type and tissue compatibility. New discoveries and future scientific advances surely await us, providing tools for assessing potential antiviral strategies and candidate vaccines against noroviruses. But before that, a more active surveillance on norovirus infections is needed.

ACKNOWLEDGEMENT

This work was supported by Operative Program “Science and Education of Intelligent Growth”, project BG05M2OP001-1.002-0001-C04.

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