

Norovirus GII.4 antibodies in the Portuguese population

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Abstract

Introduction: Norovirus GII.4 is the leading cause of outbreaks of acute and sporadic acute gastroenteritis worldwide. Information on the prevalence of norovirus in Portugal is scarce or null.

Methodology: We used a GII.4 norovirus virus-like particle-based enzyme immune assay to measure the seropositivity rate of GII.4 norovirus.

Results: A total of 342 (70%) out of 473 serum samples tested positive for anti-GII.4 norovirus IgG. No statistically significant differences were found between regions, sex and age groups.

Conclusion: Norovirus GII.4 infections are frequent in Portugal.

Key words: norovirus; antibodies; Portugal; foodborne diseases.

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Introduction

Noroviruses are the most common cause of epidemic gastroenteritis and the leading cause of foodborne outbreaks [1,2].

Noroviruses can be spread via multiple routes including fecal-oral and vomit-oral transmission [3]. Person-to-person transmission is the primary mode of spreading; and together with their environmental persistence and infectiousness, outbreaks are most frequently reported in semi-closed communities such as long-term-care facilities, hospitals, schools, restaurants and cruise ships [4].

Illness due to norovirus can occur in all age groups and clinical symptoms generally include vomiting, non-bloody diarrhea, fever, abdominal cramps, nausea, chills, myalgia and headaches; however, asymptomatic infections have also been described [4,5]. Symptoms are typically considered mild and self-limiting, lasting for 24 to 48 hours, with an incubation period of 24 to 48 hours [4]. Medical intervention is generally not required unless rapid dehydration occurs in vulnerable populations such as infants, the elderly and immunosuppressed patients, in which case fluid

therapy or additional supportive care may be needed [2,5].

Noroviruses comprise a genetically and antigenically diverse group of non-enveloped single-stranded RNA viruses classified into the family *Caliciviridae*, genus *Norovirus*. They can be grouped into at least 6 different genogroups (G) with viruses infecting humans belonging to GI, GII and GIV [5]. The majority of human infections are caused by GI and GII, and these genogroups are further subdivided into 31 genotypes [5]. In the last decade, genogroup II, genotype 4 (GII.4) noroviruses have emerged as the most frequent cause of outbreaks of acute nonbacterial gastroenteritis worldwide [6]. The high prevalence of norovirus GII.4 has been confirmed by recent seroprevalence studies in both developed and developing countries [7-9].

In Portugal, information on the prevalence of norovirus infections is scarce since these viruses are not included in the diagnostic algorithm of acute gastroenteritis in routine clinical laboratories. The lack of diagnostic testing capabilities leads to underreporting and only one confirmed foodborne norovirus outbreak has been described until today

[10]. Hence, the aim of this study was to evaluate the seroprevalence of norovirus in the Portuguese population.

Methodology

Serum samples

A total of 473 serum samples were collected in January 2012 from anonymous volunteers from Northern (n = 231), Central (n = 189) and Southern (n = 53) Portugal. Individuals enrolled in this study were healthy individuals from the University of Porto and participants of a national scientific meeting. The serum samples were from 337 females (71.2%) and 136 males (28.8%). Two hundred and forty-seven (52.2%) samples represented ages 19 to 29 years; 187 (39.5%), 30 to 39 years; 33 (7.0%), 40 to 49 years; and 6 (1.3%), 50 years and over. The study was approved by an institutional review board of the University of Porto.

Enzyme immune assay for detection of GII.4 norovirus antibodies

Sera were tested for the presence of IgG antibodies against GII.4 norovirus using an enzyme-linked immune assay (EIA) using recombinant virus-like particles (VLPs) as antigens. Briefly, norovirus GII/Hu/USA/2009/GII.4/New Orleans (GenBank accession number GU445325) VLPs were produced by cloning full-length VP1/VP2 (ORF2 and ORF3 of the genome) in a baculovirus-insect cell expression system and purified through sucrose and CsCl gradients [11]. Morphology and size of the purified VLPs was confirmed by electron microscopy.

For the VLP-based EIA, microtiter plates (96-well)

(NUNC, Milford, USA) were coated with norovirus GII.4 VLPs (1.25 µg/ml) in coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃ [pH 9.6]) and incubated overnight at 4°C. After that, incubation wells were washed three times with phosphate buffered saline (0.01 M, pH 7.4)/Tween 0.05% (PBST) and blocked with 200 µL of blocking buffer (PBS/Tween 0.05% - 10% non-fat dry milk) for 2 hours at 37°C. After washing, serum samples (50 µL) diluted in 1:1500 blocking buffer were tested in duplicate, both in VLP-coated and non-coated wells, to correct for background binding, and incubated for 1 hour at 37°C. After washing three times with PBST, the wells were incubated with goat-anti-human IgG-horseradish peroxidase that was diluted 1:12800 in blocking buffer for 1 hour at 37°C. Bound IgG was detected using TMB (Kirkegaard & Perry Laboratories Inc., Gaithersburg, USA) at room temperature for 10 minutes and the reaction was immobilized with stop solution (Kirkegaard & Perry Laboratories Inc., Gaithersburg, USA). The optical density (OD) was measured at 450 nm (MRX revelation spectrophotometer, Dynex, Magellan Bioscience). Cut-off values were determined as previously described [12]. Net absorbance (P - N) was calculated as the mean value in the antigen-coated wells (P) minus the mean value in the antigen-negative wells (N). Values with a net absorbance of 0.15 and a P/N value of > 2.0 were considered positive. A chi-square test for homogeneity of proportions (SPSS 13.0, SPSS Inc., Chicago, IL, USA) was used to evaluate differences between genders, regions, and age groups. Only values of p < 0.001 were considered statistically significant.

Table 1. Distribution of IgG anti-norovirus GII.4 seropositivity of the studied population (n = 473) according to regions of Portugal, sex and age.

Variables	Total (%)
Region of Portugal	
North	165 (71.4)
Centre	135 (71.4)
South	42 (79.2)
Sex	
Male	99 (72.8)
Female	243 (72.1)
Age (years)	
19-29	169 (68.4)
30-39	144 (77.0)
40-49	26 (78.8)
>50	3 (50.0)

Results

From the 473 serum samples, 342 (70%) tested positive for anti-GII.4 norovirus IgG. The distribution of IgG anti-norovirus GII.4 seropositivity of the studied population according to geographical region, sex and age is presented in Table 1. A slightly higher anti-GII.4 norovirus IgG seropositivity was found in people from Southern Portugal, with 79% seropositivity, compared to 71% from both Northern and Central regions of Portugal (Table 1); however, this difference was not statistically significant. No differences were observed between the seropositivity found in women and that found in men, showing 72% and 73%, respectively. Seropositivity increased with age, reaching 79% in the group aged 40-49 years.

Discussion

GII.4 norovirus antibodies were detected in 70% of the serum samples from a Portuguese cohort. Since no information on the genetic characterization of norovirus infections in Portugal was available, we selected GII.4 New Orleans antigen as this GII.4 variant emerged in 2009 and continued to cause outbreaks in 2012 [13]. Compared to data from other countries, such as France which reported a prevalence of 74.1% [14]; Italy, 91.2% [15]; and the UK, 99.5% [8], the 70% we found in the Portuguese samples was relatively low. This variance could reflect real differences in exposure levels of norovirus in different European countries, or may be the result of the fact that the VLPs used in our study were unable to detect cross-reactive antibodies against non-GII.4 norovirus strains including GI [16]. A higher seroprevalence could have been found if a Portuguese variant was used as antigen.

In conclusion, GII.4 norovirus infections frequently occur in Portugal, but they are underreported due to either the lack of knowledge among clinicians or the limited availability of norovirus diagnostic testing in routine clinical laboratories. To the best of our knowledge this is the first seroprevalence study of norovirus in the Portuguese population. Given the importance of norovirus as foodborne pathogen, diagnostic testing of stool samples for norovirus should be considered in addition to testing for routine bacterial enteric pathogens.

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References

- Hall A (2012) Noroviruses: The Perfect Human Pathogens? *J Infect Dis* 205: 1622-1624.
- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States--major pathogens. *Emerg Infect Dis* 17: 7-15.
- Lopman B, Gastañaduy P, Park GW, Hall AJ, Parashar UD, Vinjé J (2012) Environmental transmission of norovirus gastroenteritis. *Curr Opin Virol* 2:96-102
- Glass RI, Parashar UD, Estes MK (2009) Norovirus gastroenteritis. *N Engl J Med* 361: 1176-1185.
- Green KY (2013) Caliciviridae: the noroviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. 6th edition. Philadelphia: Wolters Kluwer Health/Lippincott Williams and Wilkins. p. 949-79.
- Siebenga JJ, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreiber E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M (2009) Norovirus Illness Is a Global Problem: Emergence and Spread of Norovirus GII.4 Variants, 2001-2007. *J Infect Dis* 200: 802-812.
- Nurminen K, Blazevic V, Huhti L, Räsänen S, Koho T, Hytönen VP, Vesikari T (2011) Prevalence of norovirus GII-4 antibodies in Finnish children. *J Med Virol* 83: 525-531.
- Menon VK, George S, Aladin F, Nawaz S, Sarkar R, Lopman B, Gray JJ, Gomara MI, Kang G (2013) Comparison of age-stratified seroprevalence of antibodies against norovirus GII in India and the United Kingdom. *PLoS One* 8: e56239.
- Son H, Jeong HS, Cho M, Lee J, Lee H, Yoon K, Jeong AY, Jung S, Kim K, Cheon DS (2013) Seroepidemiology of predominant norovirus strains circulating in Korea by using recombinant virus-like particle antigens. *Foodborne Pathog Dis* 10: 461-466.
- Mesquita JR, Nascimento MS (2009) A foodborne outbreak of norovirus gastroenteritis associated with a Christmas dinner in Porto, Portugal, December 2008. *Eurosurveillance* 14: pii=19355. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19355> Accessed on November 4, 2013.
- Jiang X, Wang M, Graham DY, Estes MK (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. *J Virol* 66: 6527-6532.
- Monroe SS, Stine SE, Jiang X, Estes MK, Glass RI (1993) Detection of antibody to recombinant Norwalk virus antigen in specimens from outbreaks of gastroenteritis. *J Clin Microbiol* 1: 2866-2872.

13. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinjé J (2014) Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. *J Clin Microbiol* 52:147-155.
14. Nicollier-Jamot B, Pico V, Pothier P, Kohli E (2003) Molecular cloning, expression, self-assembly, antigenicity, and seroepidemiology of a genogroup II norovirus isolated in France. *J Clin Microbiol* 41: 3901–3904.
15. Pelosi E, Lambden PR, Caul EO, Liu B, Dingle K (1999) The seroepidemiology of genogroup 1 and genogroup 2 Norwalk-like viruses in Italy. *J Med Virol* 58: 93–99.
16. Hansman GS, Natori K, Shirato-Horikoshi H, Ogawa S, Oka T, Katayama K, Tanaka T, Miyoshi T, Sakae K, Kobayashi S, Shinohara M, Uchida K, Sakurai N, Shinozaki K, Okada M,

Seto Y, Kamata K, Nagata N, Tanaka K, Miyamura Y, Takeda N (2006) Genetic and antigenic diversity among noroviruses. *J Gen Virol* 87: 909–919.

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