Journal of Clinical Virology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Genetic diversity and clinical impact of human rhinoviruses in hospitalized and outpatient children with acute respiratory infection, Argentina

Débora Natalia Marcone [a,*], Andrés Culasso [b], Guadalupe Carballal [a], Rodolfo Campos [b], Marcela Echavarría [a]

[a] Virology Unit and Clinical Virology Laboratory, Hospital Universitario CEMIC, Av. Galván 4102, Buenos Aires, Argentina
[b] Department of Virology, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 9 September 2014
Accepted 12 October 2014

Keywords:
Human rhinoviruses
Genotypes
Acute respiratory infection
Children
Molecular epidemiology

ABSTRACT

Background: Human rhinoviruses (HRV) are recognized as a cause of upper and lower acute respiratory infections (ARI). The circulating species and their clinical impact were not described in Argentina.
Objectives: To describe the molecular epidemiology of HRV in children and to determine the association of HRV species with outcome and severity.
Study design: Hospitalized and outpatients children <6 years old with ARI without comorbidities (n = 620) were enrolled (2008–2010). Demographic, clinical data and outcome were analyzed. HRV were identified by RT-PCR. Phylogenetic analysis and demographic reconstruction for HRV were performed in selected samples.
Results: HRV were detected in 252/620 (40.6%) of children; 8.5% in viral coinfection. Bronchiolitis (55%) and pneumonia (13%) were the most frequent clinical diagnosis. Of 202 inpatients with HRV: 72% required oxygen supplementation, 11% intensive care unit and 3% mechanical ventilation. HRV were identified as a risk factor for hospitalization (OR: 2.47).
All three HRV species were detected being HRV-A (55%) and HRV-C (43%) the most frequent; HRV-B was infrequent (2%). Of 44 sequenced HRV, 30 genotypes were detected. Seven of them were the most prevalent and circulated during limited periods of time. The demographic reconstruction revealed a constant population size and a high turnover rate of genotypes. Demographic and clinical outcome were similar for HRV-A and HRV-C infections.
Conclusion: This study highlights the clinical impact of HRV in children without comorbidities as a cause of lower ARI and hospitalization. The high frequency of HRV infections may be associated with the simultaneous circulation of genotypes and their high turnover rate.
© 2014 Elsevier B.V. All rights reserved.

1. Background

Human rhinoviruses (HRV) are a frequent cause of upper respiratory tract infections (URTI). However, recently, HRV were found also to be associated with lower respiratory infections (LRTI), such as bronchiolitis [1], pneumonia, exacerbation of asthma or cystic fibrosis [2,3], chronic obstructive pulmonary disease [4] and hospitalization [5].

HRV are non-enveloped positive-sense single strand RNA viruses, with a 7200-base genome, classified into three species within the *Enterovirus* genus of picornaviruses.

By phylogenetic analysis, reference serotypes have been classified in 75 HRV-A and 25 HRV-B genotypes [6] and a new species HRV-C, with 50 accepted genotypes and 13 provisionally assigned types (pat), was described in 2006 [7].

Abbreviations: ARI, acute respiratory infection; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection; HRV, human rhinoviruses; RSV, respiratory syncytial virus; Flu, influenza; PIV, parainfluenza; AdV, adenovirus; hMPV, human metapneumovirus; 5′NCR, 5′ non-coding region; IF, immunofluorescence; tMRCA, time of the most recent common ancestor; HPD, highest probability density; BSP, Bayesian skyline plot; MCC, maximum clade credibility; BA, Buenos Aires.

* Corresponding author at: Virology Unit and Clinical Virology Laboratory, Hospital Universitario CEMIC, Av. Galván 4102, Buenos Aires C1431FWO, Argentina. Tel.: +54 11 5299 0100x2375; fax: +54 11 5299 1580.
E-mail address: deboramarcone@hotmail.com (D.N. Marcone).

http://dx.doi.org/10.1016/j.jcv.2014.10.006
1386-6532/© 2014 Elsevier B.V. All rights reserved.

All 3 HRV species have a global distribution, being HRV-A and HRV-C the most prevalent; a high number of co-circulating genotypes were reported. It remains controversial whether a particular species is associated with higher severity and worse outcome.

2. Objectives

To determine the molecular epidemiology of HRV in hospitalized or outpatient children younger than 6 years old with acute respiratory infection (ARI), without comorbidities. To compare demographic, clinical data, outcome and severity among different HRV species.

3. Study design

3.1. 1-Study population

A cross-sectional, descriptive study was performed in 620 children with ARI, without comorbidities, during two consecutive years (June 1, 2008–May 31, 2010) in Buenos Aires, Argentina. Children hospitalized at either Centro de Educación Médica e Investigaciones Clínicas (CEMIC) University Hospital or Mater Dei Hospital, or outpatients attending the emergency room at CEMIC Hospital were enrolled.

Inclusion criteria were: (a) children <6 years old with LRTI and/or URTI, and symptoms onset ≤3 days; (b) informed consent form signed by parents/tutors; (c) a respiratory sample obtained at admission; (d) demographic and clinical data recorded in a specially designed form. For inpatients, the clinical course including length of stay, oxygen therapy, admission at intensive care unit and mechanical ventilation, was also recorded.

The exclusion criteria were children with cardiopathy, chronic pulmonary disease, metabolic and genetic diseases or immunosuppression.

Nasopharyngeal flocked swabs and aspirates obtained from outpatients and inpatients, respectively, were processed for rapid antigen detection by immunofluorescence (IF) for respiratory syncytial virus (RSV), adenovirus (AdV), influenza A and B (FluA, FluB), parainfluenza (PIV) 1–3 (Chemicon/Millipore) and human metapneumovirus (hMPV) (Argene).

3.2. Rhinovirus detection and typing

Viral RNA/DNA extraction was manually performed from an aliquot of the original sample stored at −70 °C, using the QIAamp MinElute Virus Spin (Qiagen), according to the manufacturer's recommendations.

HRV detection was performed by a real-time RT-PCR targeting 207 nucleotides (nt) of the 5′ non-coding region (5′NCR), using the One step RT-PCR Kit (Qiagen), primers forward (5′-CYA GCC TGC GTG GC-3′) and reverse (5′-GAA ACA CGG ACA CCC AAA GTA-3′), and a Taqman probe (5′-FAM-TCC TCC GGC CCC TGA ATG YGG C-BHQ1-3′) [8].

For HRV genotyping, an RT-PCR that amplifies 549 nt of the 5′NCR/VP4/VP2 region was performed according to a previously published protocol using primer pair 9565-reverse and 9895-forward [9]. PCR products were purified by ethanol precipitation and direct-sequenced by an automatic sequencer 3730XL (Macrogen, Korea).

To type HRV positive cases, 45 samples were selected from both, hospitalized and outpatient children, from every month of the studied period. For inpatients, we selected patients based on their length of stay at the hospital (less or more than 3 days). Strains were named BA (for Buenos Aires), followed by an identification number, a status identification (HL3 stands for hospitalized ≤3 days, HM3 for hospitalized >3 days, and A for outpatients), and the sample collection date (dd-mm-yy).

Sequences corresponding to 420 nt of the VP4/VP2 partial region, were visually inspected and manually edited with BioEdit v7.0.5.3 [10], and aligned with ClustalW v1.81 [11] using GenBank reference sequences for all HRV genotypes and human enterovirus (HEV) species as outgroup.

The obtained HRV sequences were submitted to GenBank under the following accession numbers: KF146656–KF146699.

3.3. Phylogenetic analysis

A maximum likelihood methodology was employed to study the relationship between obtained HRV sequences and reference strains. Phylogenetic trees were obtained by the heuristic search as implemented in PhyML v3.0 program [12]. The models of nucleotide substitution were selected according to the Akaike Information Criterion implemented in ModelTest 3.07 [13]. Branch support was assessed by non-parametric bootstrap (1000 pseudoreplicates). Bootstrap values greater than 70% was used to provide significant evidence for phylogenetic grouping. For recombination analysis, the sequence dataset was examined using bootscan algorithm [14].

3.4. Demographic reconstruction

A Bayesian coalescent analysis was carried out to study the relationship between the different HRV-A and C genotypes. Population dynamics and phylogeny were jointly estimated in a Bayesian framework as implemented in BEAST v1.7.1 [15]. Sampling dates were used to calibrate an uncorrelated log-normal relaxed molecular clock. Population dynamics was modeled by the non-parametric Bayesian skyline plot (BSP) [16]. Results were summarized into maximum clade credibility (MCC) trees with branches scaled in time, using Tree Annotator v1.7.1.

3.5. Statistical analysis

Results were given as percentages for discrete variables and as median, first and third quartile for continuous variables. Bi-variate associations between variables were assessed by χ^2 or Fisher exact test. Continuous variables were compared using Wilcoxon non-parametric test. The odds ratios (OR) with 95% confidence intervals (CI) were calculated. Statistical significance was assumed for p values <0.05. Statistical analysis was performed using STATA 7.0 (Stata Corp.).

4. Results

A HRV diagnosis was achieved in 252/620 (40.6%) children <6 years of age with ARI. Specifically, HRV were detected in 202/434 (46.5%) inpatients, and 50/186 (26.9%) outpatients ($p < 0.01$).

Viral antigen detection by IF was positive in 277/620 (44.7%) children. RSV was detected in 165 (26.6%), hMPV in 53 (8.5%), followed by FluA (3.4%), PIV (3.2%), AdV (2.4%) and FluB (1.9%). A viral coinfection was observed in 61 (9.8%) patients, being HRV detected in 53 (8.5%) of them. A total of 144 (23.2%) children had a negative viral diagnosis.

HRV were significantly more frequent in hospitalized patients and the presence of HRV as a single agent or in coinfection were identified as a risk factor for hospitalization: OR: 2.23 (95% CI: 1.51–3.30) and OR: 2.47 (95% CI: 1.60–4.00), respectively.

Of 252 HRV positive patients, 123 (48.8%) were <12 months, while 195 (77.4%) were <2 years old. Most were males (60.3%) and breastfed (89.7%) (Table 1). Cough was observed in 90.1% and fever in 57.8% of patients, while tachypnea, wheezing and retraction were

Please cite this article in press as: Marcone DN, et al. Genetic diversity and clinical impact of human rhinoviruses in hospitalized and outpatient children with acute respiratory infection, Argentina. J Clin Virol (2014), http://dx.doi.org/10.1016/j.jcv.2014.10.006

Download English Version:

https://daneshyari.com/en/article/6120639

Download Persian Version:

https://daneshyari.com/article/6120639

Daneshyari.com