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Characterization of human parainfluenza virus-3 circulating in Israel, 2012-2015

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ABSTRACT

Background: Human parainfluenza virus 3 (hPIV-3) causes respiratory tract infection.
Objectives: The objective of this study was to describe the epidemiology of hPIV-3 infection among hospitalized patients and characterize the circulating strains.
Study design: A cross-sectional study was conducted using respiratory samples of 15,946 hospitalized patients with respiratory symptoms in 2012–2015 in Israel. All samples were subjected to q-PCR and q-RT-PCR to determine the presence of hPIV-3 and other respiratory viruses. Samples positive for hPIV-3 were subjected to molecular typing and phylogenetic analysis.
Results: Overall, 547 samples 3.4% (95% CI 3.2–3.7) were positive for hPIV-3. Of these 87 (15.9%) were mixed infections; 41.4% with adenovirus, 40.2% with RSV (40.2%) and 19.5% influenza A viruses. The prevalence of hPIV-3 was highest (5.1%) in children aged 0–4 years. Hospitalization in oncology department was associated with increased likelihood of hPIV-3 infection: adjusted odds ratio [aOR] 2.29 (95% confidence intervals [CI] 1.78–2.96), as well as hospitalization in organ transplantation department: aOR 3.65 (95% CI 2.80–4.76). The predominant lineages were C3c (62.3%) and C1b (24.6%), followed by sub-lineages C5 (8.7%) and C3b (2.9%). A new sub-lineage emerged in our analysis, named C1d, which was 17 (1.5%) nucleotide different from C1a, 25 (2.2%) nucleotide different from C1b and 24 (2.1%) nucleotide different from C1c.
Discussion: Young children and immunocompromised patients are likely the risk groups for severe respiratory infections with hPIV-3. Strains belonging to lineages C3c and C1b, which are present worldwide, should be targeted in vaccine development. The emergence of new lineage might have public health implications and on vaccine development.

1. Background

Human parainfluenza virus 3 (hPIV-3), a memberof the *Paramyxoviridae* family, is an enveloped single stranded RNA virus with negative polarity [1]. hPIV-3 causes a spectrum of respiratory tracts infections ranging from asymptomatic upper respiratory infection in young children [2], to severe illness like bronchiolitis and pneumonia in infants [1]. hPIV-3 has also been reported to cause severe health care associated infection in newborn nurseries, bone marrow transplant and hematology-oncology units [2–5]. hPIV-3 circulates throughout the year but epidemics usually occur in the late spring and summer. Co-infections between hPIV-3 and other respiratory viruses such as respiratory syncytial virus (RSV) and adenovirus have been reported [1].

The two major antigenic proteins of hPIV-3 hemagglutinin-neuraminidase (HN) and fusion (F), were reported to be dominant targets for protective humoral immunity. The hPIV-3 HN nucleotide sequence variability allowed performing phylogenetic analysis to shed light on the different hPIV-3 variants. While hPIV-3 variants analysis is ill defined, the most recent phylogenetic analysis showed three main clusters (A, B, and C) of hPIV-3 variants with a minimal nucleotide divergence of 4.5% between clusters and average divergence of 1–2.7% within clusters. Cluster C demonstrates the most dynamic and widespread

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group that includes the majority of the variants recovered recently. Members of cluster C were further subdivided into five subclusters designated as C1-C5, with minimal nucleotide divergence of 1.9–3.1% between subclusters [6–12].

2. Objectives

The objectives of this study were to describe the epidemiology of hPIV-3 infections among hospitalized patients between 2012 and 2015 in Israel and to perform in depth molecular analysis of the HN gene of the hPIV-3 circulating variants in Israel.

3. Study design

3.1. Ethical considerations

The institutional review board of the Chaim Sheba Medical Center approved this study (Helsinki Number: 1658-14-SMC). Viral diagnostic tests were performed on the patient samples submitted to Israel Central Virology Laboratory. Residual RNA was used for molecular characterization of hPIV-3.

3.2. Patients and samples

Respiratory samples (nasopharyngeal swabs or aspirates) of 15,946 hospitalized patients presenting with respiratory symptoms were submitted to Israel Central Virology Laboratory between January 2012 and September 2015. Samples were collected using Σ-Virocult Swab and Virus Transport Medium (MWE Medical Wire, England) and transported to the laboratory within 8 h from collection. Samples were stored at 4 °C pending molecular analysis within 24 h of sample collection.

3.3. Extraction of nucleic acids

Nucleic acid was extracted from patients' samples using NucliSENS easyMAG (BioMerieux, France), according to the manufacturer's instructions. Briefly, total nucleic acid was extracted from 500 µl of well vortexes patient samples. Extracted nucleic acid was eluted in 55 µl buffer and stored at −70 °C pending analysis.

3.4. Viral detection- Real-time transcription-PCR (RT-PCR) assay

Samples were subjected to q-PCR and q-RT-PCR to determine the presence of influenza A and B viruses, influenza A H1N1pdm09, adenovirus, human metapneumovirus (hMPV) and hPIV-3 [13–15]. Real-time reverse transcription-PCR (r-RT-PCR) assay, using *Taq*Man chemistry on the ABI 7500 instrument (Life Technologies, Foster City, CA) were used. Briefly, amplification was performed in 25 µl reaction volumes, including 5 µl of sample preparation and 20 µl of one-step RT-PCR master mix (The AgPath-ID™ One-Step RT-PCR Kit, Life Technologies, Foster City, CA). Single-use viral RNA positive control set at Ct ~30 was used as a positive control for each target. The internal control RNase P was used as previously reported [16].

3.5. Molecular typing

Samples that tested positive for hPIV-3 were used to determine hPIV-3 circulating variants. The complete HN gene was amplified as previously described using OneStep RT-PCR Kit (Qiagen, Germany) according to the manufacturer's instructions [17]. Briefly, one-step RT-PCR was conducted in 25 µl reaction volume with the 10 pmol/reaction of the primer set HNS (5′-AAATCGAGTGGATCAAAATGATAAGCC-3′) and HNA (5′-TGTGTAATTGTGCTATTCTACCTTTAACG-3′). Amplified PCR products were separated on 2% agarose gel electrophoresis and visualized with ethidium bromide staining. HN RT-PCR amplified products were then purified using illustra™ ExoProStar™ 1-Step kit (GE Healthcare, UK) following the manufacturer's recommendation.

Purified HN products were diluted to 60–70 ng/µl concentration and sequenced in both directions with the primers HNS and 3HN- (5′-ACAGTGCCATTGTTAGATTCAG-3′) using ABI PRISM® BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) [6]. Reaction mixtures were then analyzed on 3500 Genetic Analyzer (Life Technology, USA).

3.6. Phylogenetic analysis

The Sequencher® 5.4 program (Gencodes Corporation, Ann Arbor, MI) was used to edit 69 complete patients hPIV-3 HN generated sequences. The obtained consensus sequences were then aligned with 65 corresponding HPIV-3 reference sequences, downloaded from the GenBank [10–12]. Sequences alignment and comparison was performed using Clustal X Neighbour Joining algorithm with 1000 boot strap values. The phylogenetic tree of the complete HN gene was visualized using NJ plot program.

3.7. Molecular evolution

To infer the evolutionary relationships and the most recent common ancestor (MRCA) for the hPIV-3 virus HN sequences, a Bayesian Markov chain Monte Carlo (MCMC) method was applied using a relaxed molecular clock (SRD06 model) in the BEAST program (version 1.7.5) to infer the time-scaled evolutionary relationships for HPIV-3. The chain length was set to 10 million. Trees were sampled every 1000 generations. The Maximum Clade Credibility tree generated by BEAST was analyzed using TreeAnnotator (v1.6.1, available at http://beast.bio.ed.ac.uk).

3.8. HPIV-3 resistance to antiviral drug Zanamivir

The two mutations (T193I and I567 V) in the HN gene that showed possible link to hPIV-3 resistance to the antiviral drug Zanamivir were checked in 69 hPIV-3 sequenced HN genes as described before [18,19].

3.9. Statistical analysis

The prevalence (in percentage) of hPIV-3 positive samples and 95% confidence intervals (CI) was calculated. The trend in hPIV-3 prevalence between 2012 and 2014 was analyzed using Cochrane-Armitage test. Seasonality analysis of hPIV-3 prevalence was performed using Freedman's test for deviation from uniform pattern, Ratchet circular scan test to detect short seasonal peaks and Hewitt's rank-sum test for longer peaks. Chi square test was used to examine differences in hPIV-3 infection prevalence according to age groups, sex and department of admission. Multivariable logistic regression model was fitted to assess adjusted associations between these variables and hPIV-3 infection. Unadjusted odds ratios (ORs) and adjusted ORs (aORs) and their 95% CIs were obtained from logistic regression models. Data were analyzed using SPSS version 23 (IBM, Armonk, New York, USA), and Winpepi[20].

4. Results

Between January 2012 and September 2015, 15,946 samples were tested. Most samples (54.4%) were collected from males. The mean age 43.1 years (standard deviation 32.4).

Overall, 547 samples tested positive for hPIV-3 by q-RT-PCR, yielding a positivity rate of 3.4% (95% CI 3.2–3.7). There was no significant difference between males and females in hPIV-3 positivity. Significant difference ($P < 0.001$) was found between age groups, with children aged 0–4 years having the highest hPIV-3 positivity (5.1%), ranging from 2.8%–3.9% in the ages 5–74, and lowest (1.7%) in the