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The Brazilian Journal of
INFECTIOUS DISEASES

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Brief Communication

Respiratory viral coinfection among hospitalized patients with H1N1 2009 during the first pandemic wave in Brazil

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ARTICLE INFO

Article history:
Received 7 August 2011
Accepted 3 October 2011

Keywords:
Influenza A H1N1 2009
Influenza coinfection
Respiratory infections

A B S T R A C T

Influenza A coinfections with other respiratory viruses were investigated in 25.8% (41/159) of the samples from patients hospitalized in 2009 at our University Hospital. Out of the 41 influenza A cases, nine cases (21.9%) were coinfected with other viruses, with a similar frequency among children and adults (p = 0.47), and seasonal influenza cases were more prevalent than H1N1 2009 influenza virus. Adenovirus was the most frequently detected (4/9) among coinfected cases. Coinfection was not associated with higher morbidity or mortality (p = 0.75).

Pandemic H1N1 2009 influenza virus had disseminated globally after being identified in Mexico and the United States in April. The H1N1 2009 morbidity and mortality were particularly severe in Brazil during the first pandemic wave. H1N1 2009 has been associated with a higher severity rate among some risk groups and young adults than seasonal influenza.[1] The frequency of viral coinfections with seasonal or pandemic Infuenza A and clinical correlation is not well known.

The purpose of this study was to investigate coinfection of confirmed influenza A and other respiratory virus in samples collected from hospitalized patients during the first pandemic wave in a Brazilian Sentinel Hospital.

We conducted a retrospective study including inpatients at the Universidade de São Paulo Hospital, São Paulo City, Brazil from August 19 to November 31, 2009. A total of 159 nasal/throat swab samples were collected from enrolled patients. Patient inclusion criteria were fever plus cough plus dyspnea plus hospitalization due to clinical suspicion of H1N1 2009 infection, according to the National Program Protocol.

Viral RNA were extracted using QIAamp Viral RNA extraction Kit (QIAGEN – Germany) and DNA using QIAamp DNA Blood Kit (QIAGEN – Germany), according to the manufacturer's instructions. Influenza A seasonal (IAV) and H1N1 2009 detections were performed following the real time protocol published by the CDC. Seasonal influenza virus B (IBV), human rhinovirus (HRV), human metapneumovirus (hMPV), adenovirus (AdV), human respiratory syncytial virus (HRSV) and human coronavirus (HCoV) detections were performed as previously described.[2-8]

Demographic, clinical, laboratory and radiologic data were obtained from medical records. Nosocomial acquisition of the H1N1 2009 was defined as an onset of illness after more than 72 hours of hospital admission.

Descriptive statistics consisted of the characterization of the studied individuals and the assessment of coinfection through calculation of the respective median value and range. Chi-squared test was used in univariate analysis comparing

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categorical variables, with a significance level of $p < 0.05$. Non-conditional logistic regression analysis was used to identify independent associations between coinfection and groups of individuals. All reported p-values are two-tailed. The dependent variable was coinfection with influenza A, and the independent variables were presence of symptomatology and groups of individuals. The results were presented as odds ratios (OR) with the respective 95% confidence interval (CI) and p-value. All data were entered into and analyzed using the Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS Inc. – Chicago, IL, USA).

Out of the 159 samples collected from hospitalized patients (Table 1), 98 were from children and 61 from adults (median age 16.4 years, range 0.33-55); there were 86 male and 73 female patients. At least one viral agent was identified in 104/159 samples (65.4%). Influenza A was the most frequently detected agent, followed by rhinovirus, human metapmeunovirus, adenovirus, HRSV and HCoV. No infections with influenza B were detected. Coinfections were observed in 15 cases as previously published.[9] Among the 15 coinfections, there were 9 cases of influenza A coinfected with other viruses and were analyzed in this study.

Influenza A infection was detected in 41/159 (25.8%) patients, 29/159 specimens (18.2%) were positive for H1N1 2009, and 12/159 (7.6 %) were positive for seasonal IAV. Viral coinfections with influenza A were detected in 9/41 (21.9%) patients, being 26% (6/23) in children and 16.7% (3/18) in adults, a difference not significant (p = 0.47; OR 1.77; 95% CI 0.37-8.31). Coinfection with other viruses was present in 4/29 (13.8%) H1N1 2009 cases and in 5/12 (41.7%) seasonal IAV cases, a difference statistically significant (p = 0.049). The risk of coinfection among IAV cases was four times higher than among H1N1 cases (OR 4.5; 95% CI 0.9-21.2). Coinfection in children was more common with IAV (4/6; 66.7%; 95% CI 22.3-95.7) than H1N1 2009 (2/6; 33.3%; 95% CI 4.3-77.7), although not statistically significant. The nine cases of multiple infections were: three seasonal IAV plus AdV, one seasonal IAV plus HRV, two H1N1 2009 plus HRSV, two H1N1 2009 plus hMPV, and one triple infection of seasonal IAV plus HRV plus AdV.

We analyzed the medical records of six coinfected patients and 27 patients monoinfected of the 41 positive patients for influenza A. 23 of the 41 positive patients were children (median age 4.7 years) and 18 were adults (median age 31.1 years), as shown on Table 1.

Two deaths were related to H1N1 2009 and no coinfection was observed in this group. Three expecting mothers were among the H1N1 2009 positive patients and one of them had a coinfection with HRSV. No deaths occurred in this group.

All patients had fever (≥ 38°C), 65% cough, 24.4% coryza, 9.7% dyspneia, 2.4% headache, and 12.2% of the

Table 1 - Characteristics and health status of 41 influenza A positive patients investigated at the Universidade de São Paulo Hospital

Characteristics	H1N1 2009	IVA	No. cases (%)
Median age, years (range)	**17.47 (0.33-55)**	**13.91 (0.5-51)**	
Gender			
Male	14	8	22 (53.7)
Female	15	4	19 (46.3)
Children	15	8	23 (56)
Adults	14	4	18 (44)
Health status			
Not available	8	0	8 (19.5)
Asthma	4	0	4 (12.2)
Others lung diseases	2	2	4 (12.2)
Diabetes	2	0	2 (6.1)
Hypertension	6	0	6 (18.2)
Cardiovascular disease	3	1	4 (12.2)
Neurological disease	2	3	5 (15.2)
Obesity	4	1	5 (15.2)
Renal disease	3	0	3 (9.1)
Anemia	3	2	5 (15.2)
HIV	1	0	1 (3)
Pregnancy	3	0	3 (9.1)
Death	2	0	2 (6.1)

children had wheezing. We did not observe differences in symptoms presented by infected patients with IAV or H1N1 2009 infections. We observed that 21.2% (7/33) positive influenza A patients were admitted to an intensive care unit: five patients had H1N1 2009, one had IAV and one had coinfection with HRSV.

In this study, from August 19 to November 31, 2009, we analyzed viral coinfections cases among hospitalized patients with confirmed influenza A. We found that coinfection with other respiratory pathogens was common (21.9%). This high rate is in accordance with other studies, where dual and multiple infections varied from 5% to 20% of all viral lower respiratory tract infection (LRTI).[10-11] Coinfections were more common among children (majority ≤ 1 year of age) than adults, and were detected most often associated with IAV than H1N1 2009. Other studies also report high rates of multiple infections in young children, mostly in children aged less than 12 months,[12] and hospitalized children with acute respiratory tract infection.[13-14] Among respiratory viruses tested in this study, AdV was the most commonly coinfecting virus with influenza A. Some studies reported high rates of coinfections with AdV. Tiveljung-Lindell et al.[15] observed dual infection with AdV in 16.2%, and Calvo et al.[16] found coinfection in 78.6% of AdV infections.

We did not observe distinct clinical characteristics of cases infected by H1N1 2009, IAV, neither there were differences between single and coinfected cases, although the number of coinfections was small. Our findings do not allow the conclusion of greater severity of coinfected cases. Papadopoulos et al.[17] reported that coinfections were associated with increased disease severity, but other studies do not support this observation. In the majority of studies, coinfections do not show clinical differences compared to single infection neither in hospitalized[18] nor in outpatient children.[10]

Establishing the relevance of coinfections is difficult. Some interpretations include concomitant infections, sequential infections, or long term, post-infection viruses.[19] The development of quantitative PCR, as well as its correlation with the clinical characteristics, may become a useful tool to clarify the role of coinfections.

These data confirm that other respiratory viruses cocirculate with influenza and suggest the need for further analysis regarding the impact of coinfections on these patients' outcome.

Acknowledgements

We acknowledge Dr. Eurico Arruda (FMRP-USP) for providing the HRV 39 isolate. The authors acknowledge the financial support of the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP Project number: 07/01166-9), and of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflict of interest

All authors declare to have no conflict of interest.

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