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Acute Respiratory Infection and Influenza-Like Illness Viral Etiologies in Brazilian Adults

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Influenza-like illness (ILI) definitions have been used worldwide for influenza surveillance. These different case definitions can vary with regard to sensitivity and predictive values for laboratory confirmed influenza. The literature has indicated the inclusion of other viruses may be the cause of these variable results. The objective of the study was to evaluate ILI national sentinel criteria and viral etiologies in adults diagnosed with acute respiratory infection (ARI) and/or ILI from 2001 to 2003 in Sao Paulo, Brazil. Clinical and laboratory evaluations were observed from 420 adults and collected on a daily basis from outpatient care units at University Hospital. The ILI definition included: fever plus at least one respiratory symptom (cough and/or sore throat) and one constitutional symptom (headache, malaise, myalgia, sweat or chills, or fatigue). DFA and RT-PCR for influenza, parainfluenza, respiratory syncytial virus, adenovirus, enterovirus, coronavirus, rhinovirus, and metapneumovirus were performed on nasal washes and 61.8% resulted positive. The respiratory viruses detected most often were influenza and rhinovirus. ILI was reported for 240/420 patients (57.1%), with influenza and rhinovirus etiologies accounting for 30.9% and 19.6%, respectively. Rhinovirus peak activity was concurrent with the influenza season. These findings highlight the implications of other viruses in ILI etiology and suggest that during the influenza season, this clinical overlap must be considered in the diagnosis and clinical management of patients. ***J. Med. Virol. 80:1824–1827, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: influenza; rhinovirus; influenza-like illness

INTRODUCTION

Influenza is characterized clinically by upper respiratory tract symptoms accompanied by fever and cough, but the clinical presentation may vary with patient age and immune status. Therefore, influenza-related illness may be difficult to distinguish on the basis of the symptoms alone from symptomatic infections caused by other respiratory viruses [Eccles, 2005].

Different influenza-like illness (ILI) case definitions have been used worldwide for influenza surveillance purposes, but the sensitivity and positive predictive value of such definitions can vary significantly depending on the co-circulation of other respiratory viruses in the community [Boivin et al., 2000].

Nevertheless, the distinction of infections caused by influenza viruses from those caused by other respiratory viruses is essential for adequate case management, as well as for monitoring effectiveness of influenza vaccination (CDC, 2007).

The purpose of this study was to evaluate the role of respiratory viruses in adults who sought medical care for acute respiratory infections (ARI), which either fit or did not fit the ILI case definition nationally adopted for clinical influenza surveillance during a 3-year period in Sao Paulo, Brazil.

METHODS

Population

The study period was from June 2001 through September 2003. Subjects were adults evaluated by general practitioners from the general community and health care workers (HCW) or outpatients of the renal transplant clinic of the Federal University of São Paulo Hospital.

NB designed the protocol; EC and AP carried out the assays; NB and EC drafted the manuscript; NB, EC, EA, AW and CG analyzed and interpreted the data.

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Inclusion Criteria and Study Design

This was a prospective study in which patients were enrolled voluntarily. Adult outpatients (≥18 years) with a clinical diagnosis of ILI and/or ARI of possible viral etiology, as seen by a physician, were considered eligible. Subjects were enrolled on a daily basis whenever a physician contacted the study team. Written informed consent was obtained before enrollment. Cases of ILI were defined by the presence of fever plus at least one respiratory symptom (cough and/or sore throat) and one constitutional symptom (headache, malaise, myalgia, sweat or chills, or fatigue). This case definition is officially recommended by the Health Office Surveillance System for the state of Sao Paulo. Patients with respiratory symptoms who did not fulfill this case definition were considered to have ARI.

Sample Collection

Enrolled patients were interviewed by a member of the study team and a nasal wash sample was collected. Nasal washes were performed according to previously published procedures [Bellei et al., 2007]. Samples were immediately transported to the virology laboratory for testing. Fresh samples were divided into three aliquots: two were frozen at −70°C for further analysis by viral isolation and PCR, while the other was centrifuged to obtain a cell pellet for DFA slides.

Respiratory Virus Assays

After centrifugation, cell pellets were spotted onto two glass slides and fixed with cold acetone (Merck, Darmstadt, Germany). Slides were then tested for the presence of influenza viruses A and B, parainfluenza viruses 1, 2, and 3, adenovirus and respiratory syncytial virus (RSV) by DFA performed according to the manufacturer's instructions (Light Diagnostics Simulfluor Respiratory Screen and Panel, Chemicon Int, Temecula, CA). In addition, frozen samples were tested by duplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza viruses A and B [Carraro et al., 2007] and a nested PCR assay for adenovirus [Allard et al., 2001]. DFA negative samples were also tested by RT-PCR-hybridization assays for rhinovirus and coronaviruses OC43 and 229E [Arruda et al., 1997], and by RT-PCR for human metapneumovirus [Falsey et al., 2003].

Epidemiological and Clinical Data

Study patients were interviewed by a study team member to obtain demographic data and information about their clinical presentation, including fever, cough, sore throat, coryza, chills and headache.

RESULTS

Patients

Nasal washes were collected from 420 cases of ARI or ILI. The patients (267 females and 153 males) were 18–83 years old (mean 35 years, median 33.5 years), and 210 of them were health care workers, 141 were patients from the general community, and 69 were kidney transplant recipients. Samples were taken 3 days (median) after the onset of symptoms on average (1–14 days), with 84% of the samples taken within the first 5 days.

Etiology

Among the 420 nasal washes collected during the study period, 260 (61.8%) were positive for a virus: 95 for rhinovirus, 49 for influenza A, 35 for influenza B, 23 for metapneumovirus, 10 for coronavirus OC43, 10 for respiratory syncytial virus, 8 for enterovirus, 7 for adenovirus, 5 for coronavirus 229E, 2 for parainfluenzavirus type 3, 1 for parainfluenza type 2, and 1 for parainfluenzavirus type 1. Fourteen (3.3%) mixed infections were detected including 5 rhinovirus plus adenovirus, 2 influenza A plus adenovirus, 1 influenza B plus adenovirus, 1 coronavirus OC43 plus adenovirus, 1 metapneumovirus plus adenovirus, 1 rhinovirus plus influenza A, 1 rhinovirus plus coronavirus OC43, 1 rhinovirus plus coronavirus 229E, and 1 influenza B plus enterovirus. RT-PCR for influenza confirmed all DFA positive cases. All adenovirus-positive samples were detected only by nested-PCR. Median age distribution of patients with positive samples is shown in Table I, and of those with virus negative samples, this age was 35 years.

Seasonal Distribution of Viruses

The rates of virus detection were not equally distributed during the study period. RSV was more frequently detected during autumn (March–May) while influenza

TABLE I. Viral Etiology-Related Distribution of Ages (Median, Years) and Symptom Frequency (%) in Adults With ARI

	Influenza (89)[a]	Rhinovirus (103)	hMPV (24)	Adenovirus (17)	RSV (10)	Coronavirus (18)	Enterovirus (9)
Age	27	31	39	34	39	40	29
Fever	91.0	50.5	54.2	58.8	60.0	55.6	55.6
Cough	85.4	80.6	79.2	88.2	80.0	72.2	77.8
Coryza	76.4	91.3	87.5	82.3	100	88.9	100
Sore throat	51.7	60.2	58.3	76.5	80.0	44.4	22.2
Headache	85.4	75.7	75.0	70.6	60.0	88.9	66.7
Myalgia	78.6	59.2	70.8	52.9	50.0	61.1	55.6
Chills	66.3	44.7	45.8	70.6	40.0	50.0	55.6

[a]Number of samples.

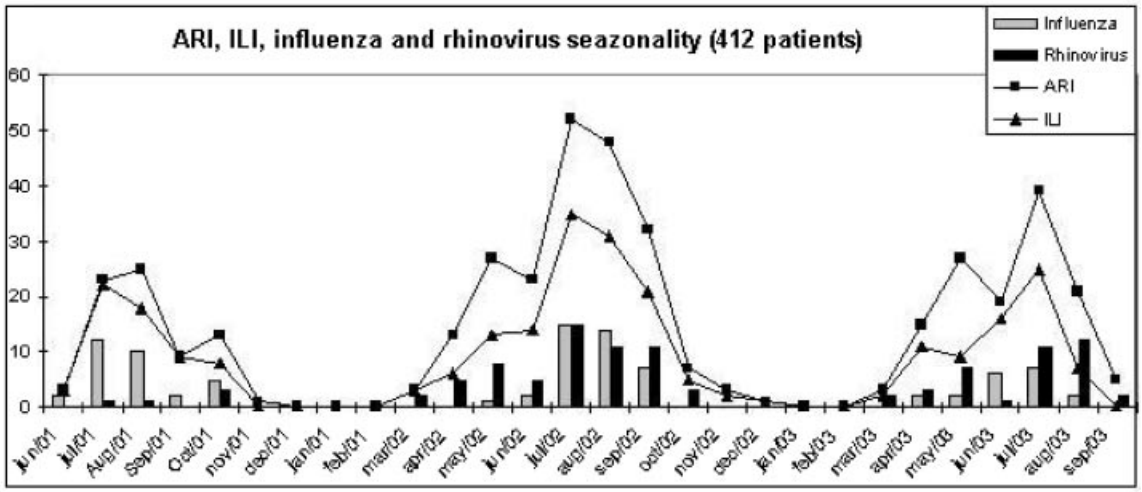
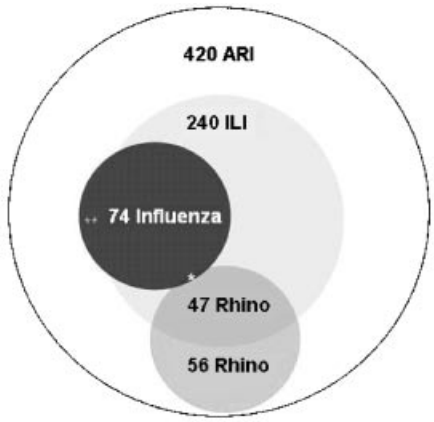


Fig. 1. Adult ARI and ILI case distribution and viral etiologies (influenza and rhinovirus) in Sao Paulo, Brazil (2001–2003).

viruses and metapneumovirus were more frequent in winter and spring (July–September). Rhinovirus was detected throughout the year, but with higher frequency coinciding with peaks of influenza activity (Fig. 1).

Clinical Data

The most frequent symptoms among all cases positive for influenza virus were fever (91%), cough (85.4%) and headache (85.4%) (Table I). Among rhinovirus positive cases, fever was reported by 50.5%, cough by 80.6% and headache by 75.7%, while coryza was present in 91.3%. A diagnosis of ILI was made in 240 (57.1%) of 420 patients with ARI. Influenza was detected in 30.9%, rhinovirus in 19.6% (Fig. 2) and other respiratory viruses in 13.7% of the ILI cases. The majority of influenza-positive cases (86.9%) presented with ILI symptoms, in contrast to 46.9% for those cases positive for rhinovirus.



*Influenza and rhinovirus co-infection detected in one patient.

** Laboratory confirmed influenza in 15 patients presenting ARI without ILI.

Fig. 2. Diagram of ARI and ILI cases, and those caused by influenza and rhinovirus.

DISCUSSION

To the best of our knowledge, this is the first study of ARI in adults in Brazil and it revealed that a viral pathogen was detected in 61.8% of the 420 samples tested in the 3 years of surveillance. This frequency of etiologic diagnosis is high, considering that not all respiratory viruses were tested and that few studies of the viral etiology of ARI in adults have found greater than 60% detection rates [Mäkelä et al., 1998; Monto et al., 2000].

The most frequently detected viral agent was rhinovirus, confirming previous findings that rhinoviruses are the most frequent causes of adult ARI in temperate regions [Arruda et al., 1997; Boivin et al., 2002; Arden et al., 2006]. However, we found that rhinoviruses circulated in Sao Paulo without the typical seasonal pattern reported in temperate regions [Arruda et al., 1997], but rather with activity peaking during the influenza season and that they were detected at higher rates than influenza viruses. This is in agreement with results by Boivin et al. [2002], who reported that rhinoviruses were frequently associated with important respiratory and systemic symptoms in adults during the influenza season in a study that recruited patients from various regions of the world (Canada, Hong Kong, Germany, Switzerland, France, United Kingdom, Norway, Finland, The Netherlands, and Belgium). However, contrary to this, a study by Louie et al. [2005] in San Francisco (USA) reported influenza detection rates twice as high as those for rhinovirus in adult ARI during the influenza season.

Influenza viruses were also common in Sao Paulo, with a circulation pattern of viruses types A and B, which is consistent with what was reported by the National Surveillance for this region of the country in the study period [Motta et al., 2006]. Overall influenza activity was considered low according to the WHO Influenza Network Surveillance [WHO, 2001, 2002, 2003]. RSV was detected mainly in autumn in the

present study, but at a lower frequency than was reported in children in another city in the same region [Cintra et al., 2001].

One important consequence of the high rhinovirus detection rate in ILI patients is the impact that this may have on assessments of vaccine effectiveness based on the occurrence of ILI cases, particularly during influenza season when rhinoviruses are also circulating. Once thought to cause only common colds, rhinoviruses have been increasingly detected in association with lower respiratory infections [Miller et al., 2007]. In line with a role of rhinoviruses in more severe clinical ARI manifestations, fever occurred in 50.5% of the rhinovirus-related cases in the present study. The presence of coryza in 91.3% of rhinovirus positive cases indicates that this is probably a good predictor of the presence of rhinovirus infection in a patient with ILI (Table I). One limitation of the present study was that the rhinovirus assay was performed only in samples that were negative for other viruses and this may have resulted in an underestimation of the true incidence of rhinovirus in this population.

Of note, all respiratory viruses investigated may cause ILI and thus contribute to the misdiagnosis of influenza cases based on clinical case definition (Table I). However, since antiviral therapy is available for influenza, etiologic diagnosis of influenza by rapid tests may be cost-effective, including reduction of unnecessary antibiotic prescriptions, decrease in hospital admissions by early flu treatment, and implementation of specific infection control precautions for influenza in hospital wards, particularly among high risk patients [Woo et al., 1997; Barenfanger et al., 2000].

ILI case definitions vary among different surveillance programs around the world [Nichol, 2006]. Sensitivity and predictive values of ILI diagnosis for confirmed influenza cases vary among studies, with positive predictive value ranging from 23% to 81% [Monto et al., 2000; Thrusky et al., 2003]. In the present study, the ILI case definition adopted by the Health Office Surveillance System was shown to be adequate for influenza strain recovery (sensitivity of 91%) from a surveillance standpoint. In contrast, this clinical case definition was not highly predictive (31%) of laboratory confirmed influenza since 69.1% of ILI cases were caused by other agents. Similar data and implications have already been reported in other studies [Thrusky et al., 2003; Navarro-Mari et al., 2005].

In conclusion, we assessed the viral etiologies of influenza-like illness in adults presenting with ARI in the largest city in South America, and the results highlight the role of other viruses, mainly rhinovirus, in patients with ILI.

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