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Temporal characteristics of respiratory syncytial virus infection in children and its correlation with climatic factors at a public pediatric hospital in Suzhou☆

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ABSTRACT

Background: Respiratory syncytial virus (RSV) is the most important viral pathogen in infants and children. It is important to analyze RSV epidemic patterns and related relevant factors in order to prevent further infections and related complications.
Objective: To explore the relationship between RSV infection rate in hospitalized children from Suzhou area and climatic factors.
Study design: 42,664 nasopharyngeal specimens from hospitalized children with acute respiratory infections were screened for RSV antigens using direct immunofluorescence. 472 RSV positive samples were randomly selected and performed real-time PCR to identify RSV subtype. Monthly meteorological data in Suzhou area was collected (average temperature, relative humidity, precipitation, total sunshine, and average wind speed) from 2001 to 2011. The relation between RSV infections and climatic factors was evaluated using correlation and stepwise regression analyses.
Results: The annual RSV infection rate in hospitalized children in Suzhou from 2001 to 2011 varied between 11.85% and 27.30%. The highest monthly infection rates occurred from November to April. The time interval from November to April was considered the infection season. Seasonal RSV infection rates from 2001 to 2010 were 40.75%, 22.72%, 39.93%, 27.37%, 42.71%, 21.28%, 38.57%, 19.86%, and 29.73%. The infection rate of any season was statistically different from the next season. There was no significant difference in RSV infection rates in the 2010–2011 and 2009–2010 epidemic seasons. Among the 472 randomly selected RSV positive samples, 412 were found to be RSV subtype A (RSV-A), while 60 subtype B (RSV-B). The monthly RSV infection rate was negatively correlated with monthly average temperature ($r = -0.84$), total sunshine ($r = -0.47$), precipitation ($r = -0.31$), relative humidity ($r = -0.20$), and average wind speed ($r = -0.20$), ($P < 0.05$). Stepwise regression analysis showed monthly average temperature fit into a linear model ($R^2 = 0.64$, $P < 0.01$) with a regression coefficient of -1.52 ($t = 15.21$, $P < 0.01$).
Conclusions: RSV infection in Suzhou occurred most frequently between November and April. The number of infections peaked every other year. Abnormally high infection rate in non-epidemic season only caused by RSV-A. Ambient temperature played an important role in the development of RSV infection.

1. Background

Respiratory syncytial virus (RSV) is the most important viral pathogen in infants and children. It spreads rapidly and is the major cause of viral pneumonia and acute bronchitis [1–5]. Some infections may progress to severe pneumonia and death [6]. It is important to analyze epidemic patterns and related relevant factors in order to prevent further infections and related complications. Large studies spanning more than 10 consecutive years have not been performed. We prospectively observed childhood RSV infections at our hospital over 11 years and correlated RSV infection with local climatic factors in order to better understand it's natural history.

2. Objective

We prospectively observed childhood RSV infections at our hospital over 11 years. Our objective was to explore the relationship

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between respiratory syncytial virus (RSV) infection rate in hospitalized children from Suzhou area and climatic factors.

3. Study design

3.1. Patient population and specimen collection and processing

This study was approved by the Ethics Committee of the Children's Hospital affiliated to Soochow University, and consent forms were signed by the parents in each case. Hospitalized children with acute respiratory infections from January 2001 to December 2011 were evaluated, and a total of 42,664 nasopharyngeal secretion specimens were collected. Among the collected samples, 42,574 had gender reported: 28,377 (66.65%) are male and 14,197 (33.35%) are female patients (male to female ratio, 1.99:1). Nasopharyngeal secretions from 3-day to 15-year-olds were collected by aspiration, using negative pressure generated by a disposable sterile suction tube inserted 7–8 cm into the nasal cavity to the pharynx. All freshly made slides with smears of exfoliated cells were used for RSV antigen detection [7]. 8275 samples were RSV positive. 8186 RSV positive patients had age recorded. 6821 (83.33%) patients were under 1 year old, 1098 (13.43%) were between 1 and 3 years old, 207 (2.53%) between 3 and 5 years old, and 60 (0.73%) > 5 years old. 8257 RSV positive patients had gender reported. There were 5788 male and 2469 female patients (male to female ratio, 2.34:1).

3.2. Direct immunofluorescence viral detection

Vital antigens were detected using the D3 UltraTM DFA Respiratory Virus Screening & ID Kit (Diagnostic Hybrids, USA). Smears of exfoliated cells were air dried followed by cold acetone fixation. 10 μl of monoclonal fluorescent antibodies against RSV was added to smears. Specimens were incubated for 30 min in the dark at 37 °C, followed by a PBS (pH 7.4) wash. Slides were examined under a fluorescence microscope (excitation wavelength = 488 nm). A slide with 5 or more RSV inclusion bodies was considered RSV positive.

3.3. Real-time PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hombrechtikon, Switzerland) that includes an on-column DNaseI (Qiagen) digest and reversed transcribed using MultiScribe reverse transcriptase and random hexamer primers (Applied Biosystems, Rotkreuz, Switzerland). Real-time PCR was performed on the ABI PRISM 7700 Sequenc-e Detector System (light cycler 480II) by using the TaqMan Gene Expression Assays for analyzing the expression of RSV-A and RSV-B (Invitrogen Biotechnology Co., Ltd.), respectively. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control gene. The primers and probe used for amplification of RSV-A, RSV-B, and GAPDH cDNA samples are following:

RSV-A: sense: 5′-AGATCACTTCTGTCATCCAGCAA-3′,
RSV-A: reverse: 5′-TTCTGCACATCATAATTAGGAG-3′,
RSV-A probe: CACCATCCAACGGAGCACAGGAGAT,
RSV-B: sense: 5′-AAGATGCAAATCATAAATTCACAGGA-3′,
RSV-B reverse: 5′-TGATATCCAGCATCTTTAAGTA-3′,
RSV-B probe: TTTCCCTTCCTAACCTGGACATA,
GAPDH sense: 5′-GAAGGTGAAGGTCGGAGTC-3′,
GAPDH reverse: 5′-GAAGATGGTGATGGGATTTC-3′,
GAPDH probe: CAAGCTTCCCGTTCTCAGCC.

Relative mRNA expression levels were calculated by the comparative threshold cycle method ($\Delta\Delta C_t$).

3.4. Collection of meteorological data

Meteorological data (monthly mean temperature (°C), relative humidity (%), precipitation, sum of sunshine, and mean wind speed (m/s)) were provided by the Meteorological Bureau of Suzhou, Suzhou City, China.

3.5. Statistical analysis

Statistical analysis was performed using SPSS20.0 software. Discreet data were analyzed using the Chi-square test. Continuous data were analyzed using the *W*-test for normality. Data with a non-normal distribution were analyzed using the Kruskal–Wallis test. The correlation between RSV infection rate and climatic factors was analyzed using a bivariate correlation analysis. The relation of climatic factors on RSV infection was analyzed using stepwise regression analysis. $P < 0.05$ was considered statistically significant.

4. Results

4.1. Overview of annual RSV epidemics

The overall RSV infection rate from 2001 to 2011 was 19.40% (8275/42,664). The annual infection rates during this period were 24.94% (715/2867), 25.83% (677/2621), 24.05% (448/1863), 25.39% (468/1843), 27.30% (276/1011), 15.55% (558/3588), 20.91% (820/3922), 13.10% (641/4892), 11.85% (608/5132), 15.06% (974/6469), and 24.72% (2090/8456), respectively. There was significant difference between annual RSV infection rates ($\chi^2 = 828.96$, $P < 0.01$).

4.2. Overview of intra-annual RSV epidemics

The annual RSV infection rates from 2001 to 2011 were calculated (Fig. 1). Infections mainly occurred from November to April (15.42–57.18%), with a peak from December to February (30.33–57.18%) and a trough from June to September (0–5.38%). A close examination on the monthly RSV infection rates from 2001 to 2011 reveals that: 2011 stands out for having significantly higher monthly infection rates from July to October than those of 2001–2010 (Fig. 2). The time period from November to April was regarded as the RSV infection season. The RSV infection rate during the 9 epidemic seasons were 40.75%, 22.72%, 39.93%, 27.37%, 42.71%, 21.28%, 38.57%, 19.86%, and 29.73%, respectively. There were significant differences in the number of RSV infections in each of the two consecutive yearly periods (corresponding χ^2 were 106.60, 94.25, 27.55, 40.84, 145.09, 111.93, 177.25, and 59.5 with all $P < 0.01$). In contrast, the RSV infection rate in the 2010–2011 epidemic season was 30.79%, which was not statistically different from 2009 to 2010 ($\chi^2 = 0.78$, $P = 0.38$). The RSV epidemic season peaked every second year. This trend was not seen after 2010. There are 472 RSV positive samples of random sampling in infection seasons from November 2006 to December 2011, RSV-A was 412 cases (87.29%) and RSV-B was 60 cases (12.71%). In epidemic seasons, a majority of detected RSV was RSV-A in 2006–2007 (93.93%), 2007–2008 (94.23%), 2010–2011 (92.98%). A majority of detected RSV was RSV-B in 2009–2010 (87.50%). On the other hand, both RSV-A (56.25%) and RSV-B (43.75%) were detected in 2008–2009. From July to December 2011, only RSV-A cases (100%) were detected (Fig. 3).

4.3. Correlation between RSV infection rate and climate

Monthly RSV infection rates from 2001 to 2011 and climatic factors were determined (Table 1). The period from November to April