Molecular Identification of Influenza, Respiratory syncytial virus And Human Metapneumovirus in Patients with Severe Acute Respiratory Infections

Thesis
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By
Nashwa Mohamed Reda Abd El Hameed Ezz El Arab
M.B.B.Ch
Cairo University.

Supervised by

Prof. Dr. Amani Ali El-Kholy
Professor of Clinical Pathology
Faculty of Medicine - Cairo University

Prof. Dr. Mona Salah El-Din Hamdy
Professor of Clinical Pathology
Faculty of Medicine - Cairo University

Prof. Dr. Iman Mohamed Abdel-Rahman
Assistant Professor of Clinical Pathology
Faculty of Medicine - Cairo University

Faculty of Medicine
Cairo University
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Abstract

Respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and influenza viruses cause severe acute respiratory tract infections in all ages especially in young children, elderly and immunocompromised. Rapid laboratory diagnosis of these viruses significantly decreases the use of antibiotics, additional laboratory testing and is associated with shorter hospitalization periods. In this study nasopharyngeal and oropharyngeal swabs were taken from patients admitted to Kasr El Aini hospitals with severe acute respiratory infections. The presence of RSV, hMPV and influenza RNA were detected using real-time reverse-transcription polymerase chain reaction (RT-PCR). Among the enrolled SARI patients, the viral etiology was identified in 64.2%. The most common respiratory virus detected was RSV constituting 41.8% of all SARI patients and 65.1% of all viruses detected, followed by hMPV that was detected in 15.7% of all SARI patients and 24.4% of all viruses detected. Adenovirus was the third most commonly detected virus representing 11.6% of all viruses detected followed by hPIV3 and hPIV1. None of cases were positive for Influenza or atypical bacteria. Mixed viral infections were detected in 11.4% of SARI patients. Adenovirus was the most frequent in co-infections. hMPV was more associated with co-infection than RSV. Co-infection between RSV and hMPV were not reported in this study.

Keywords:

Respiratory syncytial virus, human metapneumovirus, Influenza, real-time reverse-transcription polymerase chain reaction (RT-PCR), severe acute respiratory infections (SARI), Egypt
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<td>AAP</td>
<td>American academy of pediatrics</td>
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<tr>
<td>ACTB</td>
<td>Actin beta</td>
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<tr>
<td>ALRI</td>
<td>Acute lower respiratory infections</td>
</tr>
<tr>
<td>ARI</td>
<td>Acute respiratory infections</td>
</tr>
<tr>
<td>ARTIs</td>
<td>Acute respiratory tract infections</td>
</tr>
<tr>
<td>ASD</td>
<td>Atrial septal defect</td>
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<tr>
<td>AUR</td>
<td>Acute upper respiratory infections</td>
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<tr>
<td>C. pneumonia</td>
<td>Chlamyphila pneumonia</td>
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<td>CAP</td>
<td>Community acquired pneumonia</td>
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<td>CCL</td>
<td>Chemokines</td>
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<td>CDC</td>
<td>Center for disease control and prevention</td>
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<td>COID</td>
<td>Committee on infectious diseases</td>
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<td>CRP</td>
<td>C-Reactive protein</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Cycle time</td>
</tr>
<tr>
<td>CUH</td>
<td>Cairo university hospital</td>
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<td>DFA</td>
<td>Direct Fluorescent Assay</td>
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DNA………………..Deoxyribonucleic acid
ED……………………Emergency Department
EMRO……………….East Mediterranean Regional Office
FDA………………….Food and drug administration
H. influenzae ………..Haemophilus influenzae
HCo-V……………….Human coronavirus
hMPV……………….human metapneumovirus
hPIV………………..human Parainfluenzavirus
IFA………………….Immunofluorescence assay
IL……………………Interleukin
LRTI……………….Lower respiratory tract infection
M pneumonia ………..Mycoplasma pneumoniae
MCP-1……………….Monocyte chemoattractant protein 1
MIP-1………………..Macrophage inflammatory protein 1
MMR………………..Measles, Mumps and Rubella
MMWR……………….Morbidity and mortality weekly report
MR…………………..Mitral regurge
MV…………………..Mechanical ventilation
NAMRU-3………….Naval Medical Research Unit-3
NP/OP swabs..............Nasopharyngeal swabs/Oropharyngeal swabs
NRVESS..................National respiratory and enteric virus surveillance system
ORF 1....................Open reading frame 1
ORF 2....................Open reading frame 2
PCR......................Polymerase chain reaction
PDA......................Patent ductus arterioles
RANTES...................Regulated on activation, normal T cell expressed and secreted
RNA......................Ribonucleic acid
ROC......................Receiver operating characteristics
RSV......................Respiratory syncytial virus
RSVIG....................Respiratory syncytial virus immunoglobulin
RTD......................Respiratory tract disease
RT-PCR...................Reverse transcriptase polymerase chain reaction
S. aureus ..............Staphylococcus aureus
S. pneumonia............Streptococcus pneumoniae
SARI......................Severe acute respiratory infections
SCID......................Severe combined immunodeficiency
TNF......................Tumor necrosis factor
URT......................Upper respiratory tract
VSD.........................Ventricular septal defect

VTM.........................Viral transport media

WHO.........................World Health Organization
Respiratory virus infections of humans are the most common and frequent infections of man. It was estimated that 1,200 viruses are capable of infecting the respiratory tract are known to exist, although many of them are not likely to cause disease (Nolte, 2008).

In patients with pneumonia, there are no clinical criteria that suggest, with proven safety, the viral etiology. In addition, there are difficulties in establishing the general etiologic diagnosis of pneumonia, especially of viral pneumonia, which limits the knowledge about this disease and its causative agents (Treanor, 2002).

Viral pneumonias (particularly influenza pneumonia) increase the susceptibility to specific types of secondary bacterial respiratory pathogens. The role of post-viral secondary bacterial pneumonia is an important consideration. Complicated patients will need to be put on mechanical ventilation that increases the risk of nosocomial pneumonia (MMWR, 2004).

Surveillance of severe acute respiratory infections (SARI) and assessing the outcome of patients with SARI is currently of importance, as they may help identify the emergence of a new highly pathogenic infectious respiratory pathogen, and identifying risk factors for secondary bacterial infections among hospitalized SARI cases.

Prompt detection of unusual increases in the morbidity due to respiratory illness (SARI) among children and adults combined with appropriate laboratory surveillance should serve as triggers for implementing appropriate public health and hospital infection control measures to prevent, control and slow down the spread of the virus in human population.
Detection of respiratory viruses in the lab depends on viral isolation, molecular detection and detection of antigen by rapid tests. Compared to other methods polymerase chain reaction (PCR) was found to be the most reliable method for diagnosis of viruses from respiratory specimens (CDC, 2009). PCR techniques can be applied in a clinical laboratory that has no resources for viral isolation. PCR provides accurate timely results that can help in management of patients and in taking infection prevention and control precautions. However, the PCR conditions such as different RNA preparation methods, amounts of reverse transcriptase, cDNA priming strategies, dNTP concentrations, annealing temperatures (TMs), and cycling conditions should be adjusted to optimize results.

**Aim of the work**

We aimed to conduct a surveillance study (hospital-based) for severe respiratory infections with laboratory confirmation of influenza, respiratory syncytial virus and human metapneumovirus infections, to characterize the epidemiology of viral respiratory infections among SARI patients admitted to Cairo University Hospitals.
Severe Acute Respiratory Infections

Introduction:

Acute respiratory infections (ARI) cause at least six percent of the disability and death around the globe, affecting the most vulnerable populations, especially the young, the old, the ailing, and the poor.

ARI is classified based on the site of infection as Acute Upper Respiratory Infections (AURI) and Acute Lower Respiratory Infections (ALRI). AURI includes nasopharyngitis, pharyngotonsillitis and otitis. ALRI includes epiglottitis, laryngitis, laryngotracheitis, Bronchitis, Bronchiolitis and pneumonia.

AURIs are generally mild in nature and most often caused by viruses, sometimes with a bacterial component as in some cases of sinusitis and otitis media. The overwhelming majority of ARI deaths and severe illness episodes are due to ALRIs, consisting mainly of pneumonia. Nearly all severe ALRI episodes occur in children under 5 years, the elderly and immunocompromised individuals (e.g. HIV-infected). Globally, about 4.2 million ALRI deaths are estimated to occur among all age groups; of these 1.8 million are estimated to occur among children 1-59 m (Simoes et al., 2006; WHO:2008; Rudan et al., 2008).

Epidemiology:

• Incidence:

The World Health Organization (WHO) estimates there are 156 million cases of pneumonia each year in children younger than five years, Approximately one-half of them require hospital admission. In the developed
world, the annual incidence of pneumonia is estimated to be 33 per 10,000 in children younger than five years and 14.5 per 10,000 in children 0 to 16 years. (Rudan et al., 2008; Harris et al., 2011).

Hospitalization rates for pneumonia (all causes) among children younger than two years in the United States decreased after introduction of the pneumococcal conjugate vaccine to the routine childhood immunization schedule in 2000 (Fiora et al., 2009).

The overall rate of community-acquired pneumonia (CAP) in adults is approximately 5.16 to 6.11 cases per 1000 persons per year; the rate of CAP increases with increasing age (Marrie and Huang, 2005).

- Mortality:

In developing countries, respiratory tract infections are not only more prevalent but more severe than in developed countries, accounting for more than 2 million deaths annually; pneumonia is the number one killer of children in these societies (Wardlaw et al., 2006; Rudan et al., 2008).

In 2005, pneumonia and influenza combined was the eighth most common cause of death in the United States and the seventh most common cause of death in Canada. There were over 60,000 deaths due to pneumonia in the United States. Mortality is highest for CAP patients who require hospitalization, with a 30-day mortality rate of up to 23 percent in such patients. (Kung et al., 2008; File and Marrie, 2010).

- Seasonality:

Although both viral and bacterial pneumonia occur throughout the year, they are more prevalent during the colder months, presumably because direct transmission of infected droplets is enhanced by indoor crowding.
• **Risk factors:**

1. Lower respiratory tract infection (LRTI), including pneumonia, are more frequent in boys, with a male-female ratio of 1.25:1 to 2:1 (*Feigin et al.*, 2009).

2. Lower socioeconomic groups have a higher prevalence of LRTIs, which correlates best with family size, a reflection of environmental crowding.

3. Underlying cardiopulmonary disorders and other medical conditions predispose to pneumonia and contribute to increasing severity. These include:
   - Congenital heart disease
   - Bronchopulmonary dysplasia
   - Cystic fibrosis
   - Asthma
   - Sickle cell disease
   - Neuromuscular disorders, especially those associated with a depressed consciousness.
   - Some gastrointestinal disorders (e.g., gastroesophageal reflux, tracheoesophageal fistula)
   - Congenital and acquired immunodeficiency disorders (*Pelton and Hammerschlag*, 2005)

4. Cigarette smoke compromises natural pulmonary defense mechanisms by disrupting both mucociliary function and macrophage activity. Exposure to cigarette smoke, especially if the mother smokes, increases the risk for pneumonia in infants younger than one year of age. The use of cigarettes, alcohol, and other substances of abuse in adolescents may increase the risk of pneumonia by increasing the risk of aspiration through impairment of the cough and epiglottic reflexes. In addition, the use of alcohol has been
associated with increased colonization of the oropharynx with aerobic gram-negative bacilli. \textit{(Pelton and Hammerschlag, 2005)}

**Pathogenesis:**

Pneumonia occurs because of an impairment of host defenses, invasion by a virulent organism, and/or invasion by an overwhelming inoculum.

In the typical scenario, pneumonia follows an upper respiratory tract illness that permits invasion of the lower respiratory tract by bacteria, viruses, or other pathogens that trigger the immune response and produce inflammation. The lower respiratory tract air spaces fill with white blood cells (WBC), fluid, and cellular debris. This process reduces lung compliance, increases resistance, obstructs smaller airways, and may result in collapse of distal air spaces, air trapping, and altered ventilation-perfusion relationships. Severe infection is associated with necrosis of bronchial or bronchiolar epithelium \textit{(Mani and Murray, 2008)}.

**Acquisition:** The agents that cause lower respiratory tract infection (LRTI) are most often transmitted by droplet spread resulting from close contact with a source case. Contact with contaminated fomites also may be important in the acquisition of viral agents, especially respiratory syncytial virus (RSV).

Although microaspiration is the most common mechanism through which pathogens reach the lung, hematogenous spread from a distant infected site, direct spread from a contiguous focus, and macroaspiration are other mechanisms \textit{(Mason and Nelson, 2005)}.

**Virulence factors:** Some microorganisms have developed specific mechanisms to overcome pulmonary host defenses and establish infection. Examples include:
• Chlamydophila pneumoniae produces a ciliostatic factor.
• Influenza virus markedly reduces tracheal mucus velocity within hours of onset of infection and for up to 12 weeks postinfection.
• Streptococcus pneumoniae and Neisseria meningitidis produce proteases that can split secretory IgA. In addition, the pneumococcus produces other virulence factors, including: the capsule that inhibits phagocytosis, pneumolysin, a thiol-activated cytolysin that interacts with cholesterol in host cell membranes, neuraminidase, and hyaluronidase (Wunderink and Waterer, 2004; Mason and Nelson, 2005).

**Predisposing host conditions**: In addition to microbial virulence factors, diseases and conditions in the host may lead to impairment of pulmonary defense and increased risk of CAP. These conditions include:

• Alterations in the level of consciousness, which predispose to both macroaspiration of stomach contents (due to stroke, seizures, drug intoxication, anesthesia, and alcohol abuse) and to microaspiration of upper airway secretions during sleep
• Smoking tobacco
• Alcohol consumption
• Hypoxemia
• Acidosis
• Toxic inhalations
• Pulmonary edema
• Uremia
• Malnutrition
• Administration of immunosuppressive agents (solid organ or stem cell transplant recipients, or patients receiving chemotherapy)
• Mechanical obstruction of a bronchus