Prevalence of respiratory viruses in Iranian patients with idiopathic pulmonary fibrosis

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Abstract

Introduction. Idiopathic pulmonary fibrosis (IPF) is a chronic and ultimately fatal lung disease. One of the risk factors involved in the acquisition of IPF is viral infections, especially respiratory viruses. In the present study, we investigated the detection of respiratory viruses and the possible relationship between these viruses and IPF.

Methods. This cross-sectional study was supported by the Iran University of Medical Sciences (IUMS), Tehran, Iran. A total of 40 respiratory samples (five nasopharyngeal and 35 bronchoalveolar lavage specimens) were obtained from IPF patients referred to IUMS hospitals between April 2015 and December 2016. Assays were performed using the CLART Pneumovir DNA array assay, which made it possible to detect five genera of respiratory viruses simultaneously.

Results/Key findings. Altogether, 22 of the 40 participants were male. Respiratory syncytial virus (RSV), parainfluenza, rhino, corona and influenza viruses were found in 2.5% (1/40), 7.5% (3/40), 10% (4/40), 2.5% (1/40) and 0% (0/40) of cases, respectively.

Conclusion. Determining a correlation between the viruses and IPF is not an easy task, and therefore, this will require more research. In addition, the CLART Pneumovir DNA array can be considered as a useful method for simultaneous detection of several viral respiratory infections.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF), primarily occurring in older adults, is defined as a chronic and ultimately fatal lung disease which usually gets worse over time and is characterized by its progressive nature [1]. The incidence of IPF increases with advancing age, with this increase usually occurring during the fifth decade of life, and this is somewhat more frequent in males than in females [2]. The primary targets of environmental agents are alveolar epithelial cells, which are associated with IPF [3]. Although IPF is a disease of unknown etiology, several risk factors may contribute to the pathogenesis of IPF, including smoking, inhaled toxins, high body mass index and low forced vital capacity (FVC) [3, 4]. Recent studies have shown that the disorder in the immune system and its susceptibility to infection are important factors in IPF disease progression [5]. Several viruses, such as respiratory viruses, hepatitis C virus and human cytomegalovirus (CMV) can also have a role in triggering, promoting or exacerbating IPF [6, 7]. It has been shown that it is possible that epithelial cells are long-term reservoirs for persistent viral infection [8]. Cytokines and chemokines are important factors that play a key role in genesis and progression of IPF.

IL-1α and IL-1β are widely expressed cytokines by alveolar macrophages of IPF patients. This expression results in inducing a profibrotic phenotype through the synthesis of platelet-derived growth factor and procollagen types I and III [9]. Another cytokine is tumour necrosis factor alpha (TNF-α) which is produced by epithelial and endothelial cells, lymphocytes and macrophages. TNF-α is involved in different pathways including the production of IL-1, transforming growth factor beta (TGF-β), IL-6, chemokine (C-C...
motif) ligand 2, C-X-C motif chemokine ligand 8 and stimulation of cell–cell adhesion and transendothelial migration [10]. The next factor TGF-β results in modulation of extracellular matrix (ECM) production by its signaling pathway. This modulation is due to the effects different factors including fibronectin, proteoglycans, collagens I, III, IV, V and the inhibition of modifying ECM enzymes such as plasminogen, metalloproteinases and elastasthase on transcription [11].

Previous studies have shown that viruses, especially respiratory viruses, may be co-factors for the development or exacerbation of lung fibrosis [7]. One of the most prominent viral respiratory tract infections is influenza, belonging to the family Orthomyxoviridae, and some studies have shown an association between flu-infected mice, especially H5N1, and developing typical IPF during the recovery period [12, 13]. Several mechanisms may be involved in the development of lung fibrosis after influenza infection, most important of which are cytokines and chemokines [11, 14]. Previous studies have shown the level of some cytokines and chemokines including IFN-α, TNF-α, interleukins (IL-1α, IL-6, IL-10, IL-15), macrophage inflammatory proteins (MIP-1α, MIP-1β) and monocyte chemoattractant protein-1 were increased in influenza infection [15, 16]. Human parainfluenza virus (HPIV), belonging to the Paramyxoviridae family, is an important cause of upper and lower respiratory tract infection [17] with different effects on the respiratory system such as direct cell damage, allergic injury due to IgE, formation of antigen–antibody complexes, production of inflammatory mediators of the immune system, and cytotoxic T cells [18]. These effects can lead to exacerbation of IPF. Rhinovirus is a cause of common colds worldwide and belongs to the Picornaviridae family [19]. Studies have described human rhinoviruses, especially rhinoviruses type C, in patients with exacerbated fibrosis [20]. Coronaviruses belonging to the Coronaviridae family cause infections in the upper respiratory and gastrointestinal tracts of mammals and birds [21]. A positive correlation has been demonstrated between severe acute respiratory syndrome and the degree of interstitial fibrosis [22]. Respiratory syncytial virus (RSV) belongs to the family Paramyxoviridae that causes respiratory tract infections [23]. Clinical symptoms of RSV infection in adults are mainly mild symptoms often indistinguishable from common colds, but the CDC reported RSV as the ‘most common cause of bronchiolitis (inflammation of the small airways in the lung) and pneumonia in the United States’ [24]. Infection with this virus causes inflammation and obstruction of the small airways, which can be one of the reasons for pulmonary fibrosis. Smooth muscle contraction, intense peribronchial infiltration of lymphocytes and hyper-secretion of mucus are important parameters involved in airway obstruction. Apoptosis plays an important role in some lung diseases such as pulmonary fibrosis and the fusion protein of RSV triggers p53-dependent apoptosis and may be directly involved in pulmonary fibrosis [25].

The purpose of this study was to determine the prevalence of five significant respiratory virus infections – influenza, parainfluenza, rhinovirus, coronavirus and RSV – and their possible relations with IPF.

**METHODS**

**Patients and samples**

A total of 40 respiratory samples (five nasopharyngeal specimens and 35 bronchoalveolar lavage specimens) were obtained from IPF patients referred to hospitals of the Iran University of Medical Sciences (IUMS) between April 2015 and December 2016. Standard clinical criteria for IPF [26], including radiological, therapeutic, spirometry and biological data, were considered by an attending team and clinical staff. Patients with connective tissue disease, chronic hypersensitivity pneumonitis or asbestosis were excluded. The study was approved by the Ethical Committee of IUMS, Tehran, Iran.

**Nucleic acid extraction**

DNA and RNA extraction from 200 ml of NP and/or bronchoalveolar lavage (BAL) specimens was performed by QIAamp MinElute Virus Spin Kit (Cat No./ID: 57704, Qia-gen, Hilden, Germany), according to the manufacturer’s instructions. Extracted genomic DNA/RNA was stored at −70 °C before use.

**DNA array assay**

To detect the mentioned viruses in the samples, the CLART Pneumovir DNA array assay (Genomica, Coslada, Madrid, Spain) was used, according to the manufacturer’s instructions. RSV A and B; influenza viruses A, B, C; HPIV 1, 2, 3, 4A, 4B; rhinovirus (65 subtypes) and coronavirus (subtype 229E) were analysed by this method. Steps in this kit are shown below:

1. Specific fragments of the viral genome were amplified by means of two multiplex RT-PCR or PCR using biotylated primers included in the kit.
2. Detection by hybridization with specific binding probes.
3. After RT-PCR/PCR and labelling amplified products with biotin, a second step lasting 90 min using a microarray system hybridization with specific probes immobilized, was performed. Following incubation with a streptavidin-peroxidase conjugate, tetramethylbenzidine was added to the hybridization sites on the microarray. The hybridization and visualization of precipitates of the products were carried out using a microarray platform.
4. The results were automatically analysed by a microarray reader, using specific software. All data were maintained in a database and recorded as a report.

**Statistical methods**

In this cross-sectional study, all data were analysed using SPSS 21 (SPSS, IL, USA) software and displayed as the mean±SD for continuous variables or N (%) for categorical
variables. The two-sided chi-square test or Fisher’s exact test and correlation analysis were used to assess the relationships between IPF and the variables. P-values <0.05 were considered statistically significant.

RESULTS
Participants
From a total of 40 participants, 22 (55 %) were male and 18 (45 %) were female with the mean age±SD 66.6±10.65 years. All IPF patients suffered chronic pneumonia diagnosed by CT scan in recent years. The demographic information of patients is presented in Table 1.

DNA array assay
Of the 40 samples collected, nine samples were positive for the mentioned viruses. The virologic diagnosis revealed single infections in nine patients (22.5 %), and no multiple infections were found. Among single infections, RSV, parainfluenza, rhino and coronaviruses were found in 2.5 % (1/40), 7.5 % (3/40), 10 % (4/40) and 2.5 % (1/40) of cases, respectively. Influenza virus was not found.

According to the data in Table 2, significant positive associations between age with parainfluenza and rhinovirus were observed. The other associations between RSV and coronaviruses with age were not statistically significant. The associations between gender, history of fibrosis in the family, drug use, infusion and history of transplant with the respiratory viruses, were not statistically significant, except the association between rhinovirus and history of transplant (P=0.035). Further details are shown in Table 2.

DISCUSSION
Although IPF is defined as an idiopathic disease, several risk factors such as occupational and environmental exposure are involved in the disease and have been identified by epidemiologic studies. One of these risk factors can be viruses, especially respiratory viruses. These viruses may be involved in increased inflammation and play a role in IPF. The results of the current study showed 22.5 % (9/40) viral infections in IPF patients. This study was carried out for the first time in Iranian IPF patients between April 2015 and December 2016 and was conducted by the Iran University of Medical Sciences, Tehran, Iran. The role of the viruses has been widely studied in the development of respiratory symptoms and in fact, in the pathogenesis of IPF [5]. It has been shown that rhinovirus plays a significant role in the development of complications in cystic fibrosis (CF) patients and in the related pulmonary dysfunction such as reduction in FVC and forced expiratory volume in 1 s are associated with exacerbations in CF [27]. On the other hand, researchers have shown PIV in acute exacerbations of IPF is at a low level [28]. In an investigation, Wooton et al. used pan-viral microarray analysis for detection of common respiratory viral infection in 60 IPF patients. Their results showed rhinovirus, coronavirus and parainfluenza with two (3.3 %), one (1.6 %) and one (1.6 %) positive case(s), respectively [28]. In another study, Ushiki et al. analysed 14 IPF cases for detection of 12 respiratory viruses in BAL samples by the PCR method. They reported one positive sample for RSV and two positive samples for CMV; other viruses were not found [29]. Previous studies have shown that RSV infection varied from 17.4 to 30 % in children [30, 31]. Frobert et al. reported RSV infection in 35 % [32]. In the present study, RSV infection was detected in 2.5 %. The microarray is a highly sensitive method for detection of viruses. High sensitivity and broader detection of respiratory viruses are the main advantages of this method [32]. Frobert et al. performed assays using the CLART Pneumovir DNA array assay for detection of 11 genera of respiratory viruses and detected 65 % single infections in all of the cases [32]. In our study, the viral detection method was the same technique that Frobert performed while its rate of viral infection was lower than their study. However, this difference can be due to sampling, in terms of volume samples, genetic variation of the mentioned viruses and endemic virus circulation in native populations. According to previous studies in East Asia, incidence of acute IPF exacerbation has increased which it affects some of the risk factors such as smoking, exposure to the dust, endemic viruses, etc. [33]. This phenomenon could be explained by the role of environmental factors in the pathogenesis of IPF [28]. Rhinovirus was isolated in 10 % of patients and was the most commonly isolated virus in the current study.

Our study had several limitations. First, the small sample size and study were conducted in a single centre. Second, the pathogen types were specific to the study area. Thus, our results are probably not applicable to other patient populations. Third, our study ignored other possible microorganisms such as respiratory bacteria that may be involved in IPF. The association between viral infection and acute exacerbation of IPF requires further investigation.

Conclusion
Although IPF is an important complication of chronic pulmonary diseases, due to the limitations of our study, further
research is needed. The microarray is a sensitive assay and sometimes may show false positive results. Thus, it is not easy to determine the significant relevance of the positive results with IPF. In addition, a larger sample size is required for better determination of viral causes of IPF illness.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
The study was approved by the Ethical Committee of IUMS with no. 93-04-30-25391.

**References**


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**Table 2. Associations between incidence of the respiratory virus and characteristics of patients**

<table>
<thead>
<tr>
<th>Respiratory viruses</th>
<th>Age*</th>
<th>Gender†</th>
<th>History of fibrosis in the family†</th>
<th>Drug use†</th>
<th>Infusion†</th>
<th>History of transplant†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parainfluenza</td>
<td>0.57</td>
<td>2.62 (0.22–31.56)</td>
<td>0.59 (0.05–7.07)</td>
<td>1.04 (0.09–12.65)</td>
<td>1.8 (0.14–22.63)</td>
<td>0.92 (0.84–1.01)</td>
</tr>
<tr>
<td>RSV</td>
<td>0.26</td>
<td>0.95 (0.87–1.05)</td>
<td>0.95 (0.87–1.05)</td>
<td>1.08 (0.93–1.27)</td>
<td>0.97 (0.91–1.03)</td>
<td>0.97 (0.92–1.03)</td>
</tr>
<tr>
<td>Rhino</td>
<td>0.89</td>
<td>4.2 (0.44–44.4)</td>
<td>1.25 (0.16–9.88)</td>
<td>2.27 (0.28–18.27)</td>
<td>1.17 (0.1–12.8)</td>
<td>0.89 (0.8–0.99)</td>
</tr>
<tr>
<td>Corona</td>
<td>0.23</td>
<td>1.06 (0.95–1.18)</td>
<td>0.95 (0.87–1.05)</td>
<td>1.08 (0.93–1.27)</td>
<td>0.97 (0.91–1.03)</td>
<td>0.97 (0.92–1.03)</td>
</tr>
</tbody>
</table>

Bold indicates statistically significant values <0.05.
*COR (95 %) for each column.
†OR (95 %) for each column.


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