Original Article

DOI: 10.7860/JCDR/2018/34516.11786

Significant Bacterial Islolates in Patients Presenting with Acute Exacerbation of Idiopathic Pulmonary Fibrosis

Internal Medicine Section

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ABSTRACT

Introduction: Idiopathic Pulmonary Fibrosis (IPF) is a chronic and progressive, condition that is due to aberrant wound healing response following repetitive alveolar injury. Though traditionally viruses have played a key role in altering the wound healing cascade, the role played by bacteria in the pathogenesis of IPF is unclear. If there is a recurrent bacterial isolate in sputum or bronchial wash, an appropriate antibiotic both prophylactically and therapeutically can help prevent the decline in lung function and morbidity. This is particularly relevant in the treatment since it involves immunosuppressives during exacerbations.

Aim: This study analyses the sputum for bacterial isolates in patients with acute exacerbation of IPF and aims to find the significance of such association.

Materials and Methods: Sputum samples of 60 patients who came with acute exacerbation of IPF to a Tertiary Medical College and Hospital between February 2015 to March 2016 were analysed. Patients who were unable to produce sputum and who had received antibiotics for the present exacerbation prior to admission were excluded. Expectorated sputum samples were collected in wide mouth sterile containers and were subjected to gram staining and culture using blood agar after assessing the sputum quality. Chi-Square tests were used to find the significant association between the bacterial isolates in the sputum samples.

Results: Among the 60 patients, there were isolates in 78.3% (n=47) and rest had no isolates. *Streptococcus pneumoniae* was isolated in 12 patients and *Escherichia Coli* in 13 patients and these were found to have significant association statistically in patients presenting with acute exacerbation of IPF. Whereas the rest of the isolates like *Klebsiella* (n=9), *Haemophillus influenza* (n=8), *Enterococcus* (n=2) and *Moraxella catarrhalis* (n=3) were found to have insignificant association.

Conclusion: Preserving the lung functions in patients with IPF is the basis of treatment. Whether infectious causes play a role in attenuating lung function and treating these with prophylactic antibiotics needs to be seen with larger studies in different geographic areas.

Keywords: Blood agar, Lung function, Prophylactic antibiotics, Sputum

INTRODUCTION

The aetiopathogenesis of IPF remains poorly understood. The condition is thought to result from repeated episodes of alveolar injury in individuals with dysfunctional alveolar wound healing mechanism [1]. Though the repetitive alveolar injury and defective healing mechanism plays a vital part in inducing the underlying fibrosis, the environmental factors such as toxins, asbestos, cobalt may cause interstitial pneumonia and fibrosis [2]. Other agents that are known to produce diffuse lung disease include some drugs and radiation. Identified risk factors include exposure to dust, cigarette smoking, gastro oesophageal reflux disease to name a few [3].

Out of the infectious agents, bacterial exacerbation and the possible alteration of alveolar healing by bacteria are the least explored. A recent study by Molyneaux PL et al., has pointed to a disordered host defence and thus susceptibility to infection, as an important contributor to disease progression in IPF [4]. Viruses have long been suspected of playing a role in the pathogenesis of IPF and it is well recognised that many patients report a viral type prodrome preceding the initial development of respiratory symptoms [5].

Acute exacerbations are episodes of rapidly progressive, respiratory decline that occur in individuals with IPF [6]. Histologically, Diffuse Alveolar Damage (DAD) is found in acute exacerbations. Such episodes have been shown in clinical trials to affect between 4-15% of individuals with IPF per year, and are an important cause of IPF related mortality with a 3-month survival of <50% [7,8]. In individuals with IPF, infective exacerbation requiring hospitalisation confers a mortality risk indistinguishable from that seen with acute exacerbations [9]. In this study we analyse the sputum for bacterial culture in patients who came with acute exacerbation of IPF.

MATERIALS AND METHODS

This was a prospective observational study of sputum samples of 60 patients who came with acute exacerbation of IPF, in Tertiary Medical College, (Sree Balaji Medical College and Hospital, Chennai) between February 2015 to March 2016

Inclusion criteria: All patients who came with acute exacerbation of IPF, diagnosed clinico-radiologically.

Exclusion criteria: Patients who were unable to produce sputum and who had received antibiotic for the present exacerbation prior to admission.

Expectorated sputum samples were collected in wide mouth sterile containers after giving appropriate instructions for all patients. The quality of the sputum was assessed by both macroscopic and microscopic examination. All sputum samples were subjected to Gram staining and culture using blood agar.

STATISTICAL ANALYSIS

Chi-Square tests were used to find the significant association (p-value <0.05) between the bacterial isolates in the sputum samples.

RESULTS

In the study 60 patients were analysed. Among them there were isolates in 78.3% (n=47) and no isolates in 21.7% (n=13) of the subjects.

Among the isolates *Streptococcus pneumoniae* was isolated in 12 patients and *Escherichia coli (E.Coli)* in 13 patients. Upon analysis using Chi-square to test the significance of these two isolates, the p value was found to be 0.042 (p<0.05) and 0.032 (p<0.05) for these two isolates respectively, revealing a statistically significant association [Table/Fig-1,2].

E.Coli	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	34 (72.3)	47 (78.3)	0.032*
Yes	0 (0.0)	13 (27.7)	13 (21.7)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-1]: Association of *E.Coli* between isolates and no isolates.
Chi-Square: 2.929; *p<0.05

S. Pneumoniae	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	35 (74.5)	48 (80.0)	0.042*
Yes	0 (0.0)	12 (25.5)	12 (20.0)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-2]: Association of *S. Pneumoniae* between isolates and no isolates.
Chi-Square: 4.149, *p<0.05

In contrast the other isolates were *Klebsiella pneumoniae* in 9 patients (p=0.087), *Haemophillus influenza* (p=0.110), *Enterococcus* (p=0.449) and *Moraxella catarrhalis* (0.350) which were found to be statistically insignificant [Table/Fig-3-6].

Klebsiella pneumoniae	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	38 (80.9)	51 (85.0)	0.087 (N.S)
Yes	0 (0.0)	9 (19.1)	9 (15.0)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-3]: Association of *Klebsiella* between isolates and no isolates.
Chi-Square: 2.929; N.S: Not significant

H. Influenza	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	39 (82.9)	52 (86.7)	0.110 (N.S)
Yes	0 (0.0)	8 (17.1)	8 (13.3)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-4]: Association of *H. Influenza* between isolates and no isolates.
Chi-Square: 2.553; N.S: Not significant

Enterococcus	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	45 (95.7)	58 (96.7)	0.449 (N.S)
Yes	0 (0.0)	2 (4.3)	2 (3.3)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-5]: Association of *Enterococcus* between isolates and no isolates.
Chi-Square: 0.572; N.S: Not significant

M.Catarrhalis	Group			p-value
	No isolates (n=13)	Isolates (n=47)	Total	
	n(%)			
No	13 (100.0)	44 (93.6)	57 (95.0)	0.350 (N.S)
Yes	0 (0.0)	3 (6.4)	3 (5.0)	
Total	13 (100.0)	47 (100.0)	60 (100.0)	

[Table/Fig-6]: Association of *M.Catarrhalis* between isolates and no isolates.
Chi-Square: 0.873; N.S: Not significant

DISCUSSION

In a study done by Molyneaux PL et al., BAL samples were analysed from 25 patients with IPF using culture-independent metagenomic analysis [10]. Their findings were similar to us. In their study the *phylum Firmicutes (Streptococcus* and *Veillonella* species), *Proteobacteria*, and *Bacteroidetes* were most commonly encountered. The same authors were able to show by longitudinal analysis of patients with IPF in serum and Bronchoalveolar Lavage (BAL) samples that specific genes, were present in patients with IPF and such expression increased over time, supporting the theory that pathogens may provide chronic antigenic stimuli in patients with IPF [11].

In animal models there is good evidence to show that viral infection can exacerbate already existing fibrosis [12]. There is evidence to suggest a role for viruses in the pathogenesis of IPF, whereas the role of bacteria is less established. One observational evidence comes from Richter AG et al., who in 2008 demonstrated positive BAL cultures in eight of 22 stable IPF patients [13]. A large multicentre, randomized, placebo-controlled study evaluated the prophylactic use of 12 months of cotrimoxazole for IPF [14]. The authors reported that there was no difference in the primary end-point of change in vital capacity when comparing cotrimoxazole and placebo. This observation, together with the high mortality associated with bacterial respiratory tract infection in IPF, suggests that bacteria may play a role in driving IPF disease progression.

However, in a study of 43 individuals suffering an acute exacerbation of IPF, Wootton SC et al., failed to clearly identify a viral or other infectious trigger for the acute exacerbation in the vast majority of their subjects [15]. There were 43 patients with acute exacerbations of IPF and all had negative bacterial cultures and negative viral serology. Subsequent Polymerase Chain Reaction (PCR) analysis of BAL fluid identified four samples positive for rhinovirus, parainfluenza or coronavirus.

Unlike other respiratory conditions where exacerbations are truly acute events, the onset of an acute exacerbation in IPF is generally more insidious [16]. Recently, molecular culture independent techniques have identified complex microbial communities in the lower airways with distinct alterations in the microbiome occurring in a number of respiratory conditions [17,18]. In a study Friaza V et al., where *Pneumocystis jirovecii* infection was analysed in IPF, a number of uncultured bacteria in the BAL of IPF patients, using basic, culture-independent techniques were identified [19]. He also analysed the microbial flora in the BAL of 20 patients with interstitial lung diseases including idiopathic pulmonary fibrosis, non-specific interstitial pneumonia and acute interstitial pneumonia using gel electrophoresis. Both classic respiratory pathogens (e.g., *Haemophilus influenza*) and a variety of previously unrecognised or under-recognised organisms were identified. Also, interestingly a negative association was observed between the presence of *Pneumocystis jirovecii* and bacterial burden, suggesting a possible in vivo antagonism between *Pneumocystis* and bacterial species.

LIMITATION

The patients who presented with recurrent and similar bacterial isolates need to be followed up and determine whether they have a pattern of colonisation. We did not check the viral and fungal co-infections in these patients.

CONCLUSION

Thus to summarise, in IPF, the role of infectious agents in the pathogenesis and progression of IPF is unknown. Though a variety of bacterial, viral and fungus can be isolated the causal nature of these organisms in IPF remains uncertain. Whether these organisms alter the alveolar healing mechanism and alter the host's response to injury leading to accelerated decline in lung function needs to be seen with long term follow-up studies. Could treating these bacterial infections in IPF patients both with active isolates and with latent infection-prophylactically, reduce mortality and morbidity remains to be seen with a larger study in different settings.

ACKNOWLEDGEMENTS

I thank Tutors India for their statistical help in this study.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 11, 2017**
Date of Peer Review: **Feb 02, 2018**
Date of Acceptance: **Apr 21, 2018**
Date of Publishing: **Jul 01, 2018**