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Pneumocystis jirovecii colonization in Chronic Obstructive Pulmonary Disease (COPD)

Khodavaisy S[1, 2], Mortaz E[3], Mohammadi F[2], Aliyali M[4], Fakhim H[5], Badali H[6]*

[1] Department of Medical Parasitology and Mycology, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2] Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
[4] Pulmonary and Critical Care Division, Mazandaran University of Medical Sciences, Sari, Iran
[5] Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[6] Department of Medical Mycology and Parasitology/Invasive Fungi Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Corresponding author: Hamid Badali, Department of Medical Mycology & Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran;* Post Code: 48175-1665. Tel: +98 11 33543781; Fax: +98 11 33543781; Email: badalii@yahoo.com

Abstract

Chronic obstructive pulmonary disease (COPD) is associated with a chronic inflammatory response in airways and lung parenchyma that results in significant morbidity and mortality worldwide. Cigarette smoking considered as an important risk factor plays a role in pathogenesis of disease. *Pneumocystis jirovecii* is an atypical opportunistic fungus that causes pneumonia in immunosuppressed host, although the low levels of its DNA in patients without signs and symptoms of pneumonia, which likely represents colonization. The increased prevalence of *P. jirovecii* colonization in COPD patients has led to an interest in understanding its role in the disease. *P. jirovecii* colonization in these patients could represent a problem for public health since colonized patients could act as a major reservoir and source of infection for susceptible subjects. Using sensitive molecular techniques, low levels of *P. jirovecii* DNA have been detected in the respiratory tract of certain individuals. It is necessary to elucidate the role of *P. jirovecii* colonization in the natural history of COPD patients in order to improve the clinical management of this disease. In the current review paper, we discuss *P. jirovecii* colonization in COPD patients..

Keywords: *Chronic Obstructive, Pneumocystis jirovecii*, *Pulmonary Disease, Smoking*

Introduction

Chronic obstructive pulmonary disease (COPD) is a slowly progressive lung diseases characterized by airflow limitation associated with a chronic inflammatory response in both airways and lung parenchyma. It is a major cause of illness and results in significant morbidity and mortality [1]. Nowadays, COPD is the fourth leading cause of mortality among individuals, and estimated to rank third by 2020 around the world [2]. Smoking is considered as a major risk factor for the development of COPD, but not all smokers develop the disease and factors determining the severity or pattern of disease in smokers are largely unknown [3]. As a cause of systemic inflammatory response (increased levels of several circulating cytokines and acute-phase reactants, i.e., IL-8, IL-6 and TNF-α), infectious agents such as bacteria, viruses and fungi could therefore play a role in the pathophysiology of COPD [4-5]. Oxidative stress and cells likes neutrophils, macrophages and mast cells playing important role in the pathogesis of disease. *Pneumocystis jirovecii* is an atypical opportunistic fungus with lung tropism and worldwide distribution that causes pneumonia in immunosuppressed individuals such as HIV [6-7]. *P. jirovecii* colonization has been described in individuals with various lung diseases that may be important in COPD pathogenesis. Morris *et al*, 2008 believed that *P. jirovecii* colonization alone or with tobacco could be a cofactor that increase or maintain inflammatory response, thus developing COPD progression [1].

The increased prevalence of *P. jirovecii* colonization in those with COPD has led to an interest in understanding its role in the disease.

The study in a non-HIV-infected population have demonstrated that *P. jirovecii* colonization is a risk factor for severe COPD (Global Health Initiative on Obstructive Lung Disease [GOLD] Stage IV), independent of smoking history or corticosteroid abuser [8]. Thus, it is more common in subjects with severe COPD than in those with milder or no disease and is associated with a more rapid progression of disease. *P. jirovecii* colonization in COPD patients could represent a problem for public health since colonized patients could act as a major reservoir and source of infection for susceptible subjects [9]. Understanding the role of this colonization in COPD patients much has been discovered about the biology of *P. jirovecii* in understanding the role of this colonization in COPD patients. Therefore in the current review paper, we focus on *P. jirovecii* colonization with emphasis to the epidemiology, diagnosis, and treatment aspects of COPD patients.

Epidemiology

Pneumocystis pneumonia (PCP) is a potentially life-threatening infection which is the most common opportunistic infection in immunocompromised individuals and plays a role in the development of airway obstruction. Calderon et al. reported 10% of patients with chronic bronchial disease were colonized with *P. jirovecii* based on staining methods of sputum [10]. In addition in 2004, they revealed *P. jirovecii* colonization in 41% of patients with chronic bronchitis and COPD by using nested PCR technique [11]. Later, Probst et al, found that more than 21% were colonized with *P. jirovecii* in COPD by using nested PCR of various respiratory samples [12]. *P. jirovecii* colonization rate was reported, 36.7 % in the among smokers group with very severe COPD based on PCR, compared with 5.3 % of smokers with normal spirometry, mild COPD, moderate COPD and severe COPD (p= 0.004) and with 9.1% of control subjects (p= 0.007) [8].

Calderon et al. investigated 51 patients with COPD and reported 28 (55%) were colonized by *P. jirovecii* pneumonia which showed a higher level of proinflammatory cytokines than COPD without PCP [13]. Calderon *et al.*, demonstrated a strong association between *P. jirovecii* colonization and the severity of COPD. This relationship is independent of the smoking history [11]. Another study found that 16% (8/50) of COPD had *P. jirovecii* colonization by performing PCR on sputum specimens [12]. Morris et al. investigated antibodies *P. jirovecii* endoprotease kexin (anti-KEX1 antibody) in 96 patients with COPD (62.7%) whose smoking backgrounds with at least 10 packs per year. They believed that low or undetectable anti-KEX1 PCP titer among smokers might increase susceptibility to colonization with *P. jirovecii* and progression of COPD [1]. The prevalence of *P. jirovecii* colonization among COPD patients is different in published studies (Table 1).

Table 1. *P. jirovecii* colonization in COPD patients

Patients, no.	Diagnostic Sample	Diagnostic Methods	Population	% Colonization	References
50	sputum	IHC stains, IF stains	Patients with chronic bronchial disease	10.0%	[10]
8	BAL	Nested PCR	Patients with COPD	37.5%	[14]
37	BAL and sputum	Nested PCR	Patients with COPD	41%	[12]
23	BAL, sputum, and tracheal aspirates	Touch-down PCR	In-patients with suspected bacterial pneumonia and COPD	43.5%	[15]
23	BAL	Nested PCR	Patients with COPD undergoing bronchoscopy	17.5%	[16]
37	sputum	Nested PCR	Patients with chronic bronchitis	40.5%	[11]
51	Sputum	Nested PCR	COPD	54.9%	[13]
68	Lung resection	Nested PCR	COPD and other lung diseases	19.1%	[8]
50	Sputum	Nested PCR	COPD	16.0%	[17]

Abbreviation: IHC, immunohistochemical; IF, immunofluorescence; COPD, chronic obstructive pulmonary disease; PCR, polymerase chain reaction

Diagnosis

Since the diagnosis of uncultivable *P. jirovecii* is a big challenge and definitively confirmed by microscopic identification of the causative organism in sputum or bronchio-alveolar lavage by staining methods (Gomori methenamine (GMS) silver, toluidine blue-O, Giemsa staining, or Diff-Quik), or an immuno-fluorescence assay will show the characteristic cysts [18]. Monoclonal antibodies can be used to detect PCP with a rapid, sensitive, and easy-to-perform immunofl uorescence assay [19-20]. These methods are generally not adequate for detection of *P. jirovecii* colonization, and sensitive techniques is highly recommended. Fortunately with the development of diagnostic techniques, diagnoses are now established by less invasive methods and more sensitive [21-23].

Early studies of Pneumocystis surface moieties revealed a predominant surface glycoprotein, the major surface glycoprotein (Msg) [24]. Msg is encoded by a large gene family consisting of over 100 copies. Molecular examination of Msg genomic localization and expression revealed that the msg gene undergoes extensive genomic rearrangement, resulting in variations in its antigenic properties [25]. Walzer et al. developed an Enzyme-linked immunosorbent assay (ELISA) that showed a promise in diagnostic testing and epidemiologic studies [23-24]. Recent studies have supported the utility of MsgC titers as indicators of acute *P. jirovecii* pneumonia [24]. In this line it has been shown that the *P. jirovecii* protease, kexin (Kex1, Prt1) has been investigated for potential use in serologic studies [25-28]. Several studies with human subjects and experimental animal models suggest that PCP Kex may be a useful target for serologic studies and a potential target for immunologic control of PCP colonization [29]. Recently, the development of molecular detection has been instrumental in advancing the study of PCP colonization. β-

1,3-D-glucan is a polysaccharide that is present in the Pneumocystis cyst wall as well as in the walls of most fungi. It triggers an innate immune response which can be detected in BAL and serum specimens from immunocompromised patients with PCP [30]. Quantitative PCR (qPCR) assays provide a very sensitive test for detecting *P. jirovecii*. Respiratory secretions may contain low copy numbers of *P. jirovecii* in individuals who are free of pulmonary dysfunction and who are either immunologically normal or abnormal. Thus, a positive PCR test does not necessarily imply the PCP is the cause of pulmonary dysfunction. Therefore results need to be confirmed in clinical investigations under standardized protocols for specimen collection and PCR performance [31, 32].

Treatment

Despite the application of prophylaxis and therapy (trimethoprim/sulfamethoxazole) (Table 2) pneumocystis pneumonia due to *P. jirovecii* remains a life-threatening infection with significant morbidity and mortality especially in HIV-infected patients [33-37]. The second line therapies and alternatives to TMP-SMX are intravenous pentamidine, clindamycin with primaquine, dapsone with trimethoprim, atovaquone, and trimetrexate with folinic acid [38-42]. However, intravenous pentamidine is often preferred; its use is associated with a high rate of significant side effects like nephrotoxicity. Clindamycin with primaquine has excellent activity against *P. jirovecii* and the combination is second- line treatment for PCP in patients who fail treatment with TMP-SMX [43-45]. Selection of an initial anti-Pneumocystis regimen depends on the severity of the patient's illness include mild-moderate disease and moderate-sever disease. Oral TMP-SMX is more effective thanother regimens which can be given at a dose of 2 double-strength tablets (TMP 160 mg

Table 2. Treatment regimens for *P. jirovecii* colonization in COPD

First choice	Alternatives	Adjunctive corticosteroids
TMP-SMX (15–20 mg/kg TMP and 75–100 mg/ kg i.v. per day, divided q6h or q8h)	Clindamycin-primaquine, pentamidine (3–4 mg/kg i.v. per day)	Prednisone (40 mg p.o. b.i.d. 5 days, then 40 mg p.o. q.d. 5 days, then 20 mg p.o. q.d. for 11 days), methylprednisolone i.v. at 75% of prednisone dose, start at time of antibiotic initiation or at least within 72 h

and SMX 800 mg) every 8 h. In form of moderate to severe disease, intravenous therapy is preferred than oral therapy, with a dose of 15 to 20 mg/kg TMP and 75 to 100 mg/kg SMX divided every 6 to 8 h, Although, if a patient with moderate is unable to tolerate TMP-SMX, alternative choices as second- line treatment for PCP would be intravenous pentamidine or Clindamycin- primaquine (600 to 900 mg intravenously every 6 to 8 h) with primaquine (15 to 30 mg base orally daily) [46-49]. The alternative choice for mild disease include Atovaquone (750 mg orally given twice daily), dapsone (100 mg orally daily) plus TMP (15 mg/kg/day orally in three divided doses), and oral primaquine (15 to 30 mg/daily) plus oral clindamycin 300 to 450 mg every 6 to 8 h. However studies have shown that prolonged use of mono-therapy prophylaxis for *P. jirovecii* colonization might develop the drug resistancy and decreased antibiotic effectiveness [50-52]. Other agents under investigation include echinocandins. There is interest in the activity of caspofungin against the cyst form of Pneumocystis, but there is scant clinical evidence that this drug is useful for treating or preventing human disease. *Pneumocystis* spp, appear to have a biphasic life cycle consisting of an asexual phase and a sexual cycle resulting in formation of cysts. The cysts as the agent of transmission contained abundant β-1,3-D-glucan observed in the mouse model, the echinocandins are able to inhibit β -1,3-D-glucan, and reduction of cyst numbers, therefore increases the survival of mice. Thus, understanding the life cycle of this genus is crucially important to improve the managements of infections [53-54]. Although patients may worsen early during PCP treatment due to a transient inflammatory response to the organism, true treatment failure may also develop. Although drug resistance cannot be directly tested in pneumocystis, similar mutations develop in bacteria after trimethoprim-sulfamethoxazole (SXT) exposure and lead to antibiotic resistance [35]. Despite the theoretical concern for drug resistance SXT remains the treatment of choice even in those with previous sulfa exposure.

Conclusions

COPD is a major public health problem that causes chronic morbidity and mortality throughout the world. Besides, it has been recently demonstrated that colonized patients with COPD have higher systemic pro-inflammatory cytokine levels such as peripheral lymphocyte counts, IL-6, IL-8, and TNF-α, than non-colonized patients. Recent studies have shown that to pay attention on pneumocystis in the progression of chronic pulmonary diseases. Although, *P. jirovecii* colonization is a risk factor for exacerbation of COPD, it is independent of smoking history or corticosteroid usage. *P. jirovecii* colonization could provokes airflow obstruction, with the pathogen acting as a co-morbidity factor that may stimulate pulmonary inflammation and may play a pathologic role in COPD patients. Several studies suggest a potential role of *P. jirovecii* in the pathophysiology of COPD through inducing inflammatory changes with chronic lung destruction and interacting with other risks factors, i.e., tobacco or pathogenic bacteria. The presence of *P. jirovecii* in the lungs, even at low levels, may stimulate a host inflammatory response that leads to lung damage and may play a crucial role in the progression of COPD. Therefore, further investigations are highly recommended to confirm the role of *P. jirovecii* colonization in the pathogenesis of COPD to improve the clinical management.

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Authors' contributions

H.B. and S.K. design and writing the draft version of the article. E.M. and M.A. were contributed in data gathering. The rest have completed the draft and contributed in the preparation of the final article.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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