

Evaluation of sputum samples in immunocompromised hosts for *Pneumocystis jirovecii* in rural hospital at Wardha

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Abstract

Background and objectives; *Pneumocystis pneumonia* [PCP] is the most common opportunistic infection in immune compromised host [ICH] *Pneumocystis jirovecii* [*P. jirovecii*] can not be cultured in vitro and it is not a colonising pathogen therefore unambiguous diagnosis of PCP requires direct detection of cyst of organism in pulmonary samples at least by standard techniques or immune fluorescent assay. The present study is therefore undertaken with the objectives of screening of induced sputum samples in ICH for cyst of *Pneumocystis jirovecii* and to evaluate these ICH clinically who were *P. jirovecii* cyst positive.

Material and methods: This short term prospective study was conducted in the department of microbiology, JNMC Sawangi Wardha for period of 12 months [Nov 2008- Nov 2009]. Induced sputum samples from 200 ICH were screened by Giemsa staining for cyst of *P. jirovecii* in microbiology laboratory. Immuno compromised host with suspected lower respiratory tract infection were included in this study. ICH with other system involvement [GIT, CNS, skin etc] and established cases of pulmonary tuberculosis were excluded. The induced sputum samples were subjected for Gram stain and Giemsa stain. Samples were cultured on blood agar, Mac-conkey agar and chocolate agar for aerobic isolates and Sabouraud's dextrose agar for fungal isolates.

The identification of organisms was done by standard methods. If induced sputum sample was found to be positive for cyst of *P. jirovecii* then clinical evaluation of that patient in general and respiratory system examination in special was carried.

Result: Out of 200 ICH, 135 were males and 65 were females. Amongst 200 samples studied, 52 samples were positive for cyst of *P. jirovecii* [43 male and 9 female]. Clinical signs and symptoms of pneumonia were observed in 38 [73.07%] out of 52 cyst positive patients while 14 cyst positive patients showed no signs and symptoms of pneumonia. Among the bacterial isolates, non hemolytic *Streptococci* topped the list in 38 PCP positive patients. *Staphylococcus aureus* topped the list in 14 cyst positive patients with no signs and symptoms of pneumonia. Follow up of these patients in regards to treatment showed that 44 patients were successfully treated while 8 patients died of other HIV complications.

Conclusion: Induced sputum examination is safe, easy, non-invasive method of collecting sample from lower respiratory tract in immune compromised host and gives significantly positive yield of organisms. All ICH whether they show symptoms and signs of pneumonia or not, should be screened for *P. jirovecii* by induced sputum examination so that appropriate treatment or prophylaxis could be started at the earliest.

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Key words: *Pneumocystis jirovecii*, *Pneumocystis pneumonia*, induced sputum, Immuno compromised host [ICH]

INTRODUCTION:

Opportunistic infections of the lungs frequently occur in immuno compromised patients and are a major cause of morbidity and mortality. Infections reported worldwide in such patients include tuberculosis, Pneumocystis pneumonia, Cytomegalovirus pneumonia, Cryptococcosis, Aspergillosis and Candidiasis.[1,2]

Pneumocystosis is a primary disease of alveoli presenting as atypical pneumonia particularly among ICH when CD4 count falls below 250 cells/cmm. The organism was first described in 1909 by Chagas and then few years later by Delanos, who ultimately named the organism in honour of Dr. Carinii after isolating it from infected rat. Years after Dr Otto Jerovec isolated the organism from humans and the organism was renamed after him.

The taxonomic classification of the Pneumocystis was debated for some time. In 1980, biochemical analysis of the nucleic acid composition of Pneumocystis rRNA and mitochondrial DNA identified the organism as a unicellular fungus. The organism is found in three distinct morphological stages: trophozoite often exists in clusters, sporozoite [precystic form] and cyst which contains several intracystic bodies [spores].[3]

In India, there are few reports on the demonstration of *P. jirovecii* in induced sputum samples of HIV positive patients and the exact prevalence pattern of *P. jirovecii* is uncertain.[2,4,5] Moreover it is difficult to obtain the clinical samples such as broncho alveolar lavage[BAL] or trans bronchial biopsy which are invasive techniques. The present study is therefore aimed to screen the induced sputum samples in immune compromised host for the cyst of *P. jirovecii* and to evaluate ICH clinically who were *P. jirovecii* cyst positive..

Materials and methods:

This short term prospective study was conducted in the department of microbiology, JNMC Sawangi for period of 12 months [Nov 2008- Nov2009]. Induced sputum samples from 200 ICH were screened for cyst of *P. jirovecii* in microbiology laboratory.

Selection of cases

Immunocompromised host [ICH] with suspected lower respiratory tract infection were included in this

study. ICH with other system involvement [GIT, CNS, skin etc] and established cases of pulmonary tuberculosis were excluded.

Collection of sample

Sputum was induced according to the procedure of Bigby et al.[6] After a vigorous gargle with water to reduce contamination with oral debris, patients inhaled a mist of 5% hypertonic saline generated by nebuliser for 10 to 30 minutes. Patients invariably coughed vigorously during nebulisation and they were encouraged to provide as much sputum as possible during that time. If poor sample was produced, the procedure was repeated up to three times and the specimens were pooled and about 2 to 10 ml volume were collected in sterile containers.

Processing of sample

The induced sputa were processed immediately. Specimens were centrifuged for minutes at low speed and from the precipitates smears were prepared on glass slides and air dried. Smears were heat fixed [7, 8]. Gram stain was done for microbiological flora and Giemsa stain was done for cyst of *P. jirovecii* according to standard methods [9].

The smears stained by Giemsa technique were examined under oil immersion. Slides were considered negative if after 30 minutes of screening cyst of *P. jirovecii* was not seen[10]. Cyst of *P. jirovecii* are large, disc like structure 4-6µm in size, oval shaped thick walled and possessing up to eight intracystic bodies or sporozoites. The sporozoites are oval, amoeboid or peach shaped 1-2µm. In Giemsa stain, sporozoite appears as having basophilic cytoplasm and reddish purple nuclei in eosinophilic mass[3]. [fig 1]

For bacterial culture, sample was inoculated on blood and Mac-conkey agar. Chocolate agar was kept in 5-10% Co₂ in candle jar and all the plates were incubated overnight at 37°C. Sabourauds dextrose agar was inoculated for fungal isolates. Identification and conformation of isolates was done by standard procedure. [3,9]

If induced sputum sample was found to be positive for cyst of *P. jirovecii*, then clinical evaluation of that patient in general and respiratory system examination in special was carried out.

Result: The present study included 200 immuno compromised hosts.

The age of ICH range from 20 to 60 years. Out of 200 ICH, 135 were males and 65 were females. The occurrence of *P. jirovecii* cyst was seen in 52 cases [26%] amongst 200 samples studied and included 43 males and 9 females. The initial clinical evaluation on admission of patient showed that weight loss, diarrhoea, prolonged fever, lymphadenopathy and thrush were the commonest clinical presentation in these cases. Further analysis of these 52 cases for signs and symptoms of respiratory system involvement showed that non productive cough, chest discomfort, progressive exertional dyspnoea were observed in 38 cases [73.07%]. Clinical signs of pneumonia in the form of fever, tachycardia, tachypnoea and crackles in chest were seen in these 38 cases. The abnormal radiological findings in these 38 cases ranged from unilateral small, ill defined infiltrates in patients with mild or minimal respiratory symptoms [12 patients] to diffuse interstitial infiltrates in patients with severe respiratory symptoms and significant dyspnoea. [26 patients] Rest of the 14 patients who were positive for cyst of *P. jirovecii* did not show signs and symptoms of pneumonia. However out of these 14 cases, 10 cases had small ill defined localised infiltrates in radiographs suggestive of *P. jirovecii* pneumonia.

Bacterial and fungal isolates from 52 cyst positive patients [38 cyst positive patients with pneumonia and 14 cyst positive patients with no pneumonia] are shown in table 1 and 2.

Table1: Isolates from induced sputum samples of 38 *P. jirovecii* cyst positive patients

Isolates	No. of induced sputum sample
Non haemolytic Streptococci	29[76.31%]
Staphylococcus aureus	1 [2.63%]
Candida albicans	7 [18.42%]

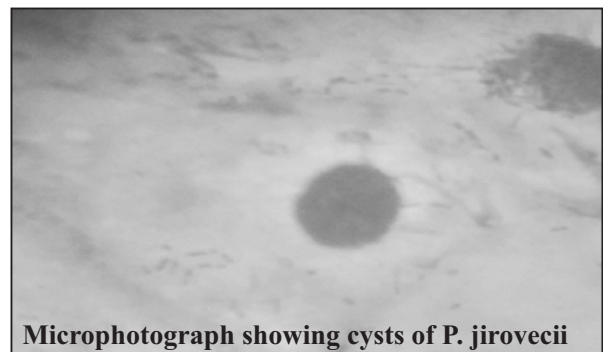
Among the isolates from 38 cyst positive patients with pneumonia, non haemolytic Streptococci [commensal] topped the list [29 samples] suggesting that *P. jirovecii* is the sole organism causing pneumonia. In 7 samples, co infection with *Candida albicans* was seen.

Table2: Isolates from induced sputum samples of 14 *P. jirovecii* cyst positive patients without pneumonia

Isolates	No. of induced sputum sample
Pure culture	
Staphylococcus aureus	2 [14.28%]
Klebsiella	1 [7.14%]
Mixed culture	
Staphylococcus aureus +	
Non haemolytic Streptococci	1 [7.14%]
Staphylococcus aureus +	
Non haemolytic Streptococci + E.coli	1 [7.14%]
Staphylococcus aureus + Non haemolytic	
Streptococci + Klebsiella	2 [14.28%]
Staphylococcus aureus +	
Candida albicans	3 [21.42%]
E. coli + Klebsiella	1 [7.14%]

Isolates from induced sputum samples of 14 *P. jirovecii* cyst positive patients with no pneumonia, *Staphylococcus aureus* topped the list in pure and mixed culture.

Follow up of these patients with regards to treatment was done. All these patients were treated with cotrimaxazole IV [20 mg/kg/day of trimethoprim and 100 mg/kg/day of sulfamethaxazole] for 21 days in two divided doses. Out of 52 cyst positive patients, 44 patients [84.61%] were successfully treated while 8 patients died of other HIV complications. The treated patients showed clinical and radiological improvement.



Microphotograph showing cysts of *P. jirovecii*

Discussion:

Though life threatening, PCP is treatable infection and hence a rapid and accurate diagnosis is mandatory.

Clinically these patients present with cough, dyspnoea, fever, abnormal chest radiograph. These signs and symptoms may also be observed in other opportunistic pulmonary infections in HIV positive patients. *P. jirovecii* can not be cultured in vitro at present and it is not a colonising pathogen, hence detection of *P. jirovecii* in pulmonary samples from patients who have not received treatment, at least by standard tinctorial or immunofluorescent assay, is currently equivalent to diagnosis of PCP.[1]

Although bronchoalveolar lavage and transbronchial biopsy gives better result, they are invasive and expensive techniques and associated with complications such as bleeding, pneumothorax, pneumonia. Bronchoscopy procedure is uncomfortable, expensive and requires specialised equipments. Therefore induced sputum sample is the best alternative, easy, non-invasive technique which could be collected with much less discomfort to the patient and is cost effective.[2,4,5,6,7,10]

In the present study, the induced sputa from 200 immunocompromised host stained by Giemsa stain revealed the presence of cyst of *P. jirovecii* in 52[26%] cases. Near equal rate of detection of cyst of *P. jirovecii* in induced sputum by Giemsa stain was seen in various studies. Usha MM et al [10] had obtained 28.12% positivity, Mishra et al [4] 25.3%. Pitchenik et al [5] and Bigby et al [6] had obtained higher positivity rate i.e. 55% and 56% respectively. Though positivity rate with other stains such as Gomorie's methamine silver [GMS] and toluidine blue is higher, the use of more than one staining technique does not increase the diagnostic yield.[4] In the study by Mishra et al[4], out of 279 PCP positive samples, positivity rate by GMS was 100%, toluidine blue 98.2% and Giemsa 91.77%. They inferred that though Giemsa stain is less sensitive, it is simple, easy to perform and economical and it may be used in screening and GMS stain be reserved for confirmation of doubtful cases.

Among the isolates from 38 cyst positive patients with pneumonia, non haemolytic Streptococci [commensal] topped the list [29 samples] suggesting that *P. jirovecii* is the sole organism causing pneumonia. In 7 samples, co infection with *Candida albicans* was seen.

Isolates from induced sputum samples of 14 *P.*

jirovecii cyst positive patients with no pneumonia, *Staphylococcus aureus* topped the list in pure and mixed culture. Our findings correlate with Usha et al.[10]

All 52 patients received cotrimaxazole therapy. Clinical and radiological improvement was seen in 44 patients while 8 patients died of other complications.

Hence it is concluded that induced sputum examination is safe, easy, non-invasive method of collecting sample from lower respiratory tract of ICH patients and gives significantly positive yield of organisms. All the HIV positive patients whether they show symptoms and signs of pneumonia or not, should be screened for *P. jirovecii* by induced sputum examination so that appropriate treatment or prophylaxis could be started at the earliest.

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VAPICON

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