ORIGINAL RESEARCH PAPER
Volume-9 | Issue-1 | January-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

MYCOLOGICAL PROFILE OF LOWER RESPIRATORY TRACT SAMPLES- A STUDY FROM NORTH INDIA

Microbiology

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ABSTRACT

INTRODUCTION: Opportunist fungal infections are on the rise due to a changing health care Paradigm. Since the data of systemic fungal infections from our valley are scant, we conducted this study to evaluate the prevalence of fungal infections in chronic lower respiratory infections not resolved by antibacterial chemotherapy and to observe the mycological profile from lower respiratory tract samples of such patients..

MATERIALS AND METHODS: This was a retrospective study conducted for a period of 4 years from January 2015 to Jan 2019 in the Department of Microbiology, Government Medical College Srinagar. A total of 221 lower respiratory tract samples (148- bronchoalveolar lavage, 49- sputum, and 22-pleural fluid and 2-endo bronchial biopsy samples) were received from patients with suspected chronic non-resolving lower respiratory tract infections from Chest Disease Hospital, Srinagar. Specimens were processed for detection of bacteria, fungi and mycobacteria as per standard methods.

RESULTS: The most common host factors in our study population were patients with post primary pulmonary tuberculosis lesions (cavitary) {n=70}, chronic obstructive pulmonary disease {n=20}, bronchiectasis {n=10}, non-resolving consolidation {n=70}, and uncontrolled diabetes mellitus {n=50}.The commonest co-morbid condition associated was diabetes mellitus. The male to female ratio was 1.2:1.Out of 221 samples, 97(44.29%) tested positive for fungal elements. Sixty-five percent of fungi isolated were *Aspergillus* spp. Among these, *Aspergillus fumigatus* was found to be the most common species isolated (16%). *Scopulariopsis* spp.(6.5%) and *Rhizopus* spp (5%) were other molds grown on culture.

CONCLUSION: Patients with respiratory tract symptoms not responding to conventional treatment should be screened for fungi by microscopy and culture in addition to other diagnostic modalities.

KEYWORDS

Respiratory, Fungi, Aspergillus

INTRODUCTION

Globally opportunist fungal infections are on the rise. A higher number of cases have been diagnosed post-mortem, indicating an under-estimate of the prevalence of infection worldwide.[1]India being the most populous country of the world, is also experiencing an upward trend in invasive fungal diseases. Among the Invasive fungal diseases, fungal lung infections are the most common. The increased prevalence of fungal lung infections is largely attributed to increased numbers of immune-compromised , heightened awareness of these infections and subsequently high index of clinical suspicion in suspected patients, confirmed by improved laboratory methods for the diagnosis of such infections.[2-4] Because the data of fungal respiratory tract infections from our valley are scant, we conducted this study to evaluate the prevalence of fungi in suspected chronic lower respiratory infection samples, not resolved by antibacterial chemotherapy and studied their mycological profile.

MATERIALS AND METHODS

This was a retrospective study conducted for a period of 4 years from January 2015 to Jan 2019 in the Department of Microbiology, Government Medical College Srinagar. All lower respiratory tract samples (Sputum, Bronchoalveolar lavage-(BAL), Pleural fluid and Endo bronchial biopsy) received from patients with chronic non-resolving lower respiratory tract ailments from a chest disease hospital Srinagar were evaluated . All work was performed in a biological safety cabinet with careful consideration to prevent contamination.

Specimens were processed for detection of bacteria, mycobacterium and fungi as per standard methods.[5]

Volume of samples received was about 5-10 ml .BAL & Pleural fluid samples were centrifuged at 1500g for 10 minutes Clot or membranous material if present was minced with scalpel and combined with concentrated fluid. Dense fluids were thinned with sterile distilled water before centrifugation.. The resulting pellet was used for microscopy (Direct KOH mount, ZN Staining, Wet mount and Gram staining) and culture. Sputum samples were digested with N –acetyl –L-cysteine before inoculation. For fungi, each sample was cultured on Sabouraud Dextrose Agar (with gentamycin) and Potato Dextrose Agar. The inoculated specimens were incubated at 37°C and 25°C Temperature for four weeks.

Yeast-form fungi were identified according to standard clinical laboratory methods, including Gram staining, germ tube testing, Chromagar identification and the Dalmau plate technique. Mould-form fungi were identified using colony morphology, microscopic findings and slide culture techniques.

This study was exempted from ethical clearance..

RESULTS.

The Average age group of patients from which maximum numbers of samples were received was 40-60 yrs of age. The male to female ratio was 1.2:1.Maximum number (n=150) of samples received were of patients with rural background {farmers, stone quarry workers, construction labourers}.There was no high risk patient of bone marrow transplantation or haematological malignancy in our study group.

The patients included in the study were those with lower respiratory tract ailments with high index of suspicion for fungal infection. They were patients with post-primary pulmonary tuberculosis cavitatory lesions (n=50), chronic obstructive pulmonary disease (n=71), bronchiectasis (n=10), non-resolving consolidation (n=70) or slow to resolve lung infiltrates non -responding to antibacterial therapy (n=20). Diabetes mellitus was the commonest co –morbid condition in patients with these respiratory illnesses (n=50).

A total of 221 non -repetitive lower respiratory tract samples were received. Out of these 148 were bronchoalveolar lavage (BAL), 49 sputum, 22-pleural fluid and 2 were endo bronchial biopsy samples).

Of the total samples received, 97 (43.9%) tested positive for fungal elements. The percentage positivity for fungi was found to be high (n=28; 57.1%) in sputum as compared to that in pleural fluid (n=3; 13.63%). 43.5% of BAL samples (n=64) were positive for fungal elements (Table 1).

Out of the fungus positive samples, 40.6% of BAL (n=26), 53.5% of sputum (n=15) & 66% of pleural fluid (n=3) samples were positive on both direct KOH microscopy and culture .39.5% of BAL (n=23) & 39% of sputum (n=11) samples were positive on culture only.

Culture positivity was highest in sputum (92%), followed by BAL fluid

(75.6%). Out of three pleural fluid samples only two grew fungi (66%). (Table 2)

23% of BAL samples & 0.07% of sputum samples were positive on direct KOH microscopy but negative on culture. Both the endobronchial samples were only positive on direct KOH microscopy (Table 2).

Among the culture positive samples (n=77), Aspergillus spp. was the most common fungus (64.9%; n=50) grown, followed by Candida Albicans (24%; n=23).Scopulariopsis spp. and Rhizopus were grown in 6.5 %(n=7) and 5% (n=5) of the samples respectively.

Among the Aspergilli, Aspergillus fumigatus was the most common fungus grown on culture (n=12; 23%).

DISCUSSION

Opportunistic fungal infections, in comparison to endemic mycoses, are the common causes of fungal pneumonias in developing countries such as India.[7]This is especially true in a rising population with co-morbid conditions such as uncontrolled diabetes, cancer immunotherapy, tuberculosis and diabetic keto acidosis.[2,8] In the present study, 43% of lower respiratory tract samples were positive for fungi, in patients with chronic non-resolving respiratory infections. Sripriya et al.[11]isolated fungi from 29.9% of bronchial wash specimens in a study from south India. Overall, fungal positivity (KOH & culture) in bronchoalveolar lavage was lower (40.6%) than in sputum samples(53.5%)(Table 1).This could be attributed to the fact that sputum samples were sent from admitted patients in an advanced stage of infection where the initial diagnosis was most likely missed. Similar results were documented by Rouhani et al.[9] {58.3% from sputum and 32% from BAL}. Although commensal yeasts and environmental inhalation of spores are important causes of false culture positivity in sputum samples.

Direct KOH examination was a valuable tool in BAL and endobronchial biopsy specimens (Table 2). Overall, 23.4% (n=15)of BAL samples, 33%(n=1)of pleural fluid samples and both endobronchial biopsy samples showed fungal elements (Hyphae, , mycelia, spores, yeast cells) on direct KOH microscopy but failed to grow on culture. This could be attributed to the patients were already receiving fluconazole for treatment. We suggest that KOH microscopy in clinically suspect cases should always be carried out early before start of any therapy (anti bacterial/anti fungal/anti mycobacteria).

The fungal culture profile of the isolates grown showed that sixty-five percent of the total isolates were (Table 3) *Aspergillus* spp. *Aspergillus* is known to cause myriad pulmonary presentations, which include aspergilloma, allergic broncho-pulmonary aspergillosis, allergic alveolitis, invasive aspergillosis, chronic necrotizing aspergillosis and mere colonization of the respiratory tract.[3,10]As in other studies[4,8-11], we found that the *Aspergillus* group is a common fungal isolate of lower respiratory tract specimens. We believe, as suggested by Nalesniket al.[12] such results should not be overlooked as mere contamination by clinicians. Among the Aspergilla, *Aspergillus fumigatus* was the most common fungus isolated (24%).followed by Aspergillus flavus(20%).Apart from these, many other species i;e, *A.terreus, A.nidulans, A.glaucus* and *A.versicolor*) grew on culture. However Aspergillus species (44%) other than Aspergillus fumigatus comprised a majority of isolates. This is in contrast to the studies done

elsewhere . Non-aspergillus moulds (S*copulariopsis*=6.5% and *Rhizopus*=5%) isolated in our study pose another alarming aspect of fungal infections which should not be overlooked. *Scopulariopsis* has been reported as a fungal pathogen in lung transplant patients[13]and preformed pulmonary cavities in recent years,[14] and we believe its role in other clinical settings, such as those related to post tuberculosis cavitary lesions or lung malignancies, should be further studied.

Economy of this region is primarily agriculture and horticulture based .The use of anti fungals in horticulture is increasing .Anti fungal susceptibility testing which was not carried by us owing to limited resources ,does demand focus of research in our part of the world.

As has been highlighted by many studies worldwide[15,16] patients with mucormycotina isolations from pulmonary samples along with corroborative clinical findings should be taken seriously and managed with anti-fungal treatment.

CONCLUSION

Aspergillus species are the common fungal pathogens of the respiratory tract in our part of the world. All patients with respiratory symptoms, especially with underlying co morbid conditions, should be screened for fungal elements by microscopy and culture at the start of any therapy.

Acknowledgments; the authors are highly grateful to Mrs. Badakhshan Andrabi for her technical support.

Conflict of interest

None

Table 1. Specimen-wide distribution.

Sample No.	Sample	Total no of samples	Positive for fungal elements N (%)
1	BAL	148	64 (43.24)
2	Sputum	49	28 (57.1)
3	Pleural fluid	22	3 (13.63)
4	Endobronchial biopsy	2	2 (100)

Table 2. Distribution of positive samples with KOH and culture positivity.

	BAL	Sputum	Pleural fluid	Endobronchial biopsy
Only Culture+	23	11	0	0
Only KOH+	15	2	1	2
Both+	26	15	2	0
Total	64	28	3	2
Culture positive	49	26	2	0

Table 3. Mycological profile of culture positive samples.

Sample No.	Fungi	BAL	PLEURAL FLUID	SPUTUM	Total N (%)
1	*Aspergillus spp.*	17	0	7	24 (31)
2	*A.fumigatus*	5	1	6	12 (16)
3	*A.flavus*	7	0	3	10 (13)
4	*A.terreus*	2	0	0	2 (2.6)
5	*Aspergillus versicolor*	2	0	0	2 (2.6)
6	*Candida albicans*	12	0	6	18 (23)
7	*Scopulariopsis*	2	1	2	5 (6.5)
8	*Rhizopus*	2	0	2	4 (5)
	Total	49	2	26	77

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