ISSN 2394–806X (Print), ISSN 2454-5139 (Electronic)
IJHRMLP, Vol: 06 No: 01, January, 2020
Printed in India

ORIGINAL RESEARCH PAPER

Concomitant fungal infections in patients of pulmonary tuberculosis attending respiratory medicine OPD

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Received on November 03, 2019; editorial approval on December 23, 2019

ABSTRACT

Introduction: *Fungal infections are frequently encountered in clinical practice; especially the incidence of concomitant fungal infection has been increasing among immunocompromised patients. Present study was carried out to identify of fungal pathogens from sputum sample of pulmonary tuberculosis patients and to assess and determine the prevalence of different fungal pathogens in pulmonary tuberculosis patient.* ***Materials and methods:*** *A total of 100 pulmonary tuberculosis patients were interviewed using pre-tested questionnaires and direct mount and culture of sputum was performed for each.* ***Results:*** *Of 100 patients, the commonest were in the age group 31-40 years (n = 29, 29%) followed by age group 41-50 years (n = 22, 22%). Majority of participants were tea garden workers (n = 36, 36%) followed by daily wage workers (n = 14, 14%). It was observed that 26% (n = 26) were KOH positive and culture positive; 1% (n = 1) was KOH negative and culture positive; 30% (n = 30) were KOH positive and culture negative; 43% (n = 43) were both negative. Highest co infection was with C. albicans (n = 15, 68.18%) followed by C. tropicalis (n = 4, 18.18%). Prevalence of mycotic co infection was highest in Multi-drug Resistant TB (MDR-TB) (60%) followed by Category II (35.71%) than Category I (19.40%) DOTS recipients. The prevalence of fungal infection in male smokers was found to be statistically significant (P<0.05).* ***Conclusion:*** *Mycotic confection in patients with pulmonary tuberculosis is inevitable. Adequate measures need to be taken for the accurate identification and treatment of these opportunistic infections, which are associated with high rates of morbidity and mortality.*

Keywords*: Candida albicans; immunocompromised patients; multi-drug Resistant TB; opportunistic infections; smoking.*

INTRODUCTION

According to WHO, Tuberculosis (TB) is one of the top ten causes of death worldwide. In 2018, an estimated 10 million people fell ill with TB and 1.5 million died from the disease.[1] Six countries account for 60% of the total, with India leading the count. It is a leading killer of HIV-positive people. Ending the TB epidemic by 2030 is among the health targets of the newly adopted Sustainable Development Goals.[2] Malnutrition, overcrowding, poor air circulation and poor sanitation have increased the risk of TB in the lower socio-economic strata of the society.[2] When such predisposing factors are superimposed with fungal infections, the prognosis of the disease deteriorates gravely. With the increase in the number of immunocompromised patients, incidence of life- threatening fungal infections has also increased significantly.[3,4] Pulmonary mycosis

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Cite this article as: *Deka Bhakhita, Borgohain Parismita, Barua Purnima. Concomitant fungal infections in patients of pulmonary tuberculosis attending respiratory medicine OPD. Int J Health Res Medico Leg Prae 2020 January;6(1):58-62. DOI 10.31741/ijhrmlp.v6.i1.2020.12*

superimposed on tuberculosis influences treatment and has high mortality. As of 2015, Assam registered maximum number of tuberculosis cases in Northeast India, out of which the tea garden community is the most affected.[5] The prognosis of the disease is further deteriorated with the superadded fungal infections and widespread appearance of multi-drug resistant TB.[6-8] The aim of this study is to record the concomitant fungal infection in patients with pulmonary tuberculosis attending Respiratory Medicine OPD in Jorhat Medical College and Hospital. Most of the times these fungal infections are not diagnosed and often mistaken for recurrence of tuberculosis.[9] These opportunistic infections if diagnosed early can be treated effectively to improve the prognosis of the disease and successful implementation of the drug treatment. Therefore, present study was carried out to identify, assess and determine the prevalence of different fungal pathogens in pulmonary tuberculosis patients.

MATERIAL AND METHODS

A cross sectional observational study was carried out in the Department of Microbiology and Out Patient Department (OPD) of Pulmonary Medicine, Jorhat Medical College and Hospital, Jorhat from 20th July-20th September 2017. A total of 100 patients were taken considering a nonresponse rate of 10%. All patients were enrolled based on proper inclusion criteria and exclusion criteria after taking informed consent.

Inclusion criteria

1. Patient with age group > 15years and in case of < 15 years, consent was taken from guardian.
2. Patients who gave informed consent to participate in the study or who were willing to participate.
3. Diagnosed cases of pulmonary tuberculosis and those receiving treatment
4. Patients who are diagnosed with HIV, diabetes or any other immunocompromised state.

Exclusion criteria

1. Participants not willing to give consent.

Project proposal and the consent form along with the peer reviewed questionnaire were approved by the Institutional Ethics Committee (Human) of Jorhat Medical College, Jorhat, Assam (Ref No. SMEJ/JMCH/MEU/841/2011/3106).

Collection of sample

A detailed sociodemographic data was collected along with history of on-going anti-tubercular therapy based on RNTCP guideline 2016[10] with the help of a pre-designed and pretested questionnaire. A written informed consent was obtained after explaining the purpose and the scope of the study. The participants were given sterile containers for collection of sputum. An early morning sputum and one spot sputum sample was collected. After proper instruction early morning sputum was collected before rinsing mouth or intake of food and water. The spot samples were collected in the Pulmonary Medicine OPD or ward under supervision after rinsing mouth with water. After collection, the samples were immediately transported to the Microbiology department for further laboratory investigation. In case of delay, the samples were refrigerated at 4°C.

Maintaining all biosafety precautions the sample was subjected to treatment with 10% Potassium Hydroxide (KOH) for 24 hours placed in a moistened petri plate and subsequently observed under the microscope for the presence of any fungal element and a part of the sample was cultured onto Sabouraud Dextrose Agar and Mycosel Agar in duplicate (Hi-media India Ltd.) and kept at room temperature.

Pulmonary candidiasis was diagnosed based on the presence of budding yeast cells along with pseudohyphae in direct 10% KOH mount, Germ tube test, growth on Chrom Agar and Cornmeal agar. Moulds on SDA and Mycosel Agar were subjected to lactophenol cotton blue mount and slide culture. Molecular characterization of the moulds were carried out in Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India by sequencing of internal transcribed spacer (ITS) region of rDNA. Yeast were identified by Matrix-assisted Laser desorption/ionization Time of flight (MALDI-TOF) (Bruker Daltonics. Bremen, Germany).

Data Analysis

The collected data was noted in a systematic tabular form. The calculations were done using MS Excel and SPSS version 25.0.

RESULTS

In this study, out of 100 enrolled patients, 62% (n = 62) were female and 38% (n = 38) were male. The age range of 31-40 years were mostly affected (n = 29, 29%) followed by age group 41-50 years (n = 22, 22%). Majority of participants were tea garden workers (36%) followed by 14% daily wage and businessmen each.

Out of 100 samples collected, 26% (n = 26) were KOH positive and culture positive; 1% (n = 1) sample was KOH negative and culture positive; 30% (n = 30) were KOH positive and culture negative; 43% (n = 43) were both negative (**Table 1**).

Table 1 Results of direct microscopic examination using KOH microscopy and fungal culture

Isolates	Culture +ve	Culture –ve
KOH+ve	26(26%)	30(30%)
KOH-ve	1(1%)	43(43%)

In the study, 22% (n = 22) of the coinfection was with *Candida* spp. followed by 1% (n = 1) *Aspergillus niger*, 1% (n = 1) *A. terreus*, 1% (n = 1) *Mucor* sp. and 1% (n

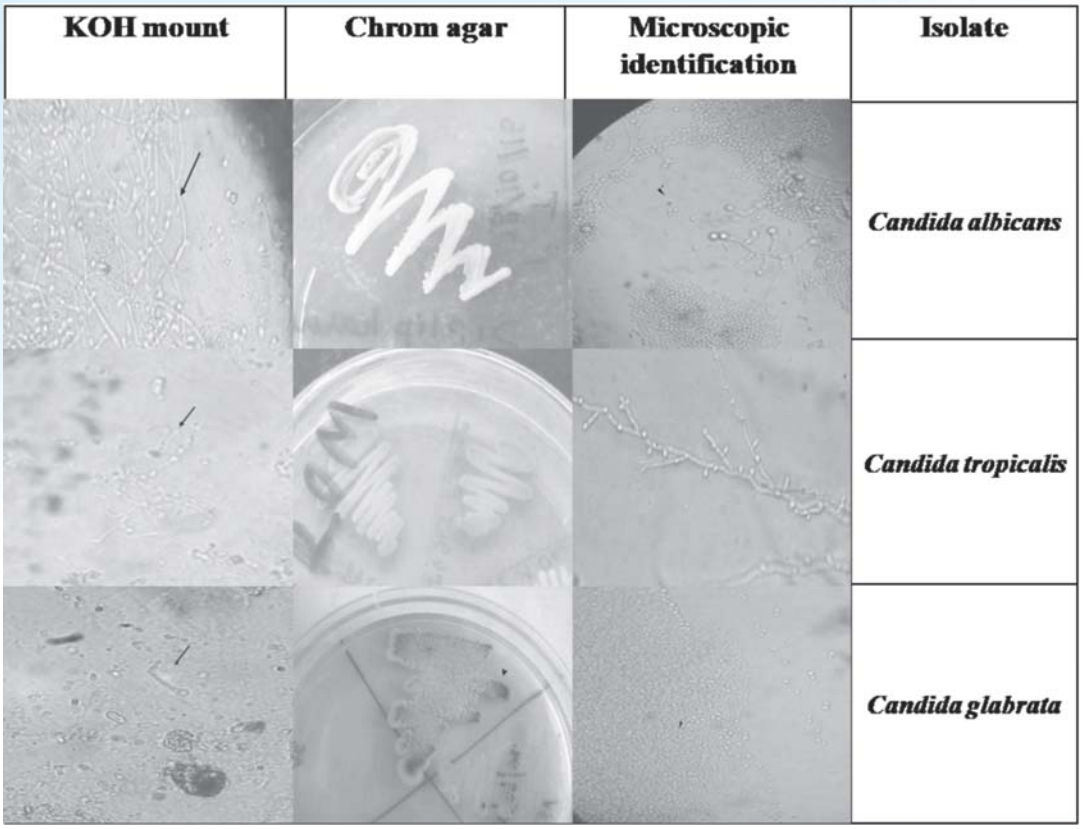

Figure 1 Photographs from direct mount and culture of *Candida* spp.

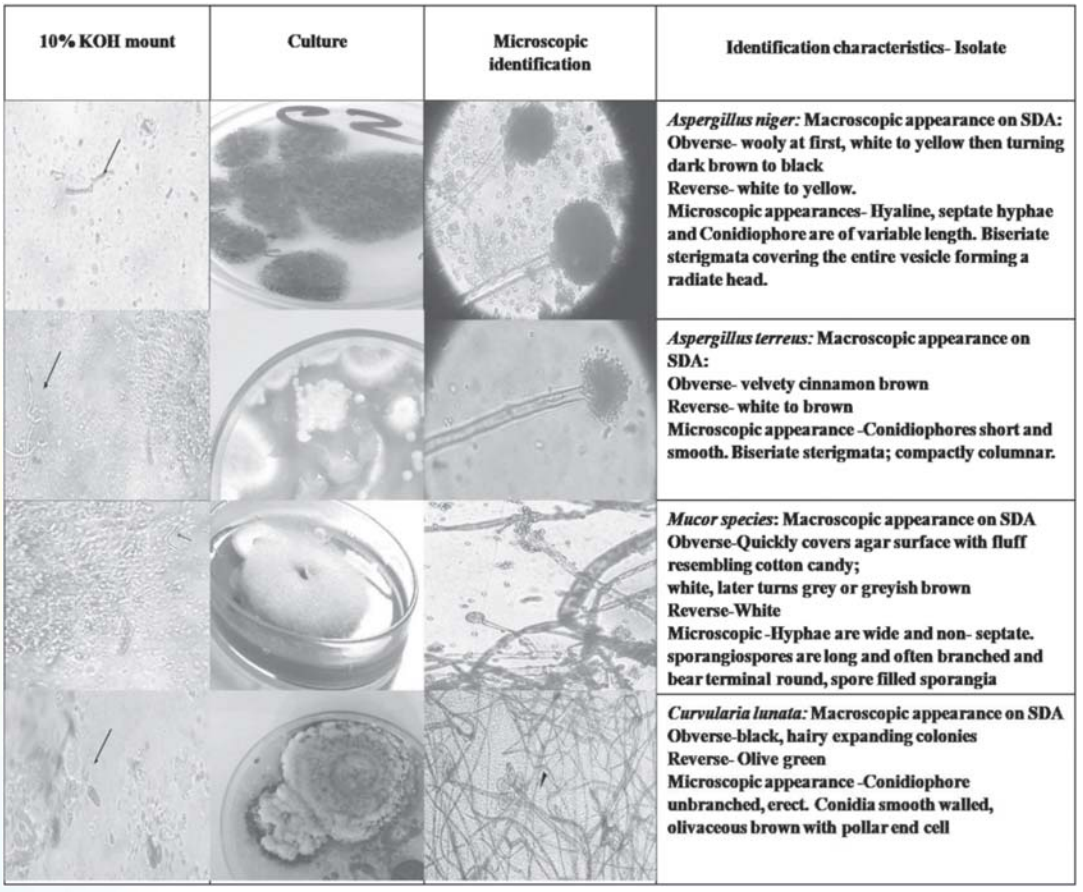

Figure 2 Direct mount and culture of moulds

= 1) *Curvularia lunata* (**Figure 1** and **2**). Among *Candida* spp., highest co infection was with *Candida albicans* (68.18%, n = 15) followed by *Candida tropicalis* (18.18%, n = 4) and *Candida glabrata* (13.63%, n = 3).

The prevalence of mycotic coinfection was highest (60%) in MDR-TB followed by Category II (35.71%) and Category I (19.40%) DOTS recipients (**Table 2**). Out of 26 patients suffering from superadded fungal infection, 13 participants were undergoing DOTS category I treatment, 10 with DOTS category II and 3 of them were taking treatment for MDR TB.

Table 2 Distribution of population suffering from fungal infections based on the category of DOTS (n = 100)

Fungi	DOTS category			Total
	Category I (67)	Category II (28)	MDR TB(5)	
Candida spp.	12	8	2	22
Aspergillus terreus	-	1	-	1
Aspergillus niger	-	-	1	1
Mucor sp.	-	1	-	1
Curvularia lunata	1	-	-	1
Total	13/67 (19.40%)	10/28 (35.71%)	3/5 (60%)	26

The highest co-infection was seen in tea garden workers (46.15%, n = 12) followed by retired workers (15.38%, n = 4) and businessman (15.38%, n = 4) each. Of 26 patients suffering from superadded fungal infection, the male manual workers (57.69%, n = 15) had high preponderance of acquiring superadded fungal infection, the male manual workers (57.69%, n = 15) had high preponderance of acquiring superadded fungal infection. It was also observed that 11.53% (n = 3) male suffering from diabetes along with pulmonary tuberculosis were found to have concomitant fungal infection. The prevalence of fungal co infection was significant ($P < 0.05$) among the male smokers (58%, n = 15).

DISCUSSION

Fungal infection associated with pulmonary tuberculosis is a complicated health condition among TB patients. The present study detected 26% prevalence of concomitant fungal infection with pulmonary tuberculosis. *Candida* spp. (22%) shows highest coinfection, similar observation of 26% co-infection with *Candida* spp. was made by Baradhkar et al.[11] According to the study of Kali et al., 2013 prevalence of *Candida* co-infection of lung ranges between 15-32%.[12] *Candida* forms a part of normal microbial flora of healthy individuals. When the host resistance is lowered, these commensals turn into aggressive pathogens causing life threatening systemic infections. The role of *Candida* spp. as secondary invaders in patients having pre-existing diseases like pulmonary tuberculosis is well documented.[13]

Filamentous saprophyte fungal pathogen and their spore have a wide distribution in the air and the transmission of infection occurs always through inhalation.[13] *Aspergillus* is one of the airborne fungi that do not normally cause disease, but immunocompromised patients are more susceptible of acquiring these infection.[13] In this study, the predominant *Aspergillus* spp. were *A. terreus* (1%) and *A. niger* (1%). This is in contrast to those obtained by Razmpa et al.[15] where 30% of *A. flavus* was documented. These variations in the incidence and isolation of various fungi can be attributed to geographical variations of the conducted studies[13]. *Mucor* sp. was isolated from 1% of cases in our study. Mucormycosis is an opportunistic infection caused by ubiquitous filamentous fungus. Pulmonary involvement with *Mucor* spp. occurs in severe immunosuppression.[15]

Present study showed that the prevalence of concomitant fungal infection is higher in MDR-TB (60%) ollowed by Category II (35.71%). This is similar to the study by Mathavi et al.[17] where fungal infection was more common in category II patients than category I. In recent studies it has been observed that patients with MDR-TB show low IFN-c production when compared with patients with non-resistant tuberculosis before and after treatment which can be attributed to the development of mycotic co-infection in these patients.[8]

In our study the highest (46.15%) prevalence of fungal co-infection was in the tea garden workers. According to the study of Chelleng et al.[5] the preponderance of the tea garden community towards pulomonary tuberculosis was associated to a state of low body mass index, poor living conditions, illiteracy and irregular income. The acquisition of fungal infection could be possibly due to their constant exposure to the fungal ecosystem in the tea garden. The climatic condition of Jorhat and north eastern region which temperature, rain and humidity provides an excellent niche for various fungal infections in the North Eastern region.[18]

CONCLUSION

The study brings into light the burden of co fungal infections amongst the patients suffering from pulmonary tuberculosis especially amongst the tea garden workers. The living and working conditions superadded by the lowered immune status impacts the susceptibility of the individual to fungal infection. Hence, adequate measures need to be taken for the early identification and treatment of these opportunistic infections, which are associated with high rates of morbidity and mortality along with improving their living standards.

Acknowledgments: This study was supported by Short Term Studentship- Indian Council of Medical Research (STS- ICMR) scheme, Govt. of India (Reference ID: 2017-01536). We would like to thank Dr. Shivaprakash M Rudramurthy, Professor, Dept. of Medical Microbiology, PGIMER for the technical support in molecular identification

of the isolates. We are also very grateful to Department of Pulmonary Medicine and Department of Microbiology, Jorhat Medical College and others who assisted us in the implementation of this research.

Author's contributions: We declare that this work was done by all the authors named in this study with equal contributions.

Conflict of interests: No conflict of interest.

Ethical clearance: Obtained.

Financial disclosure: We are very grateful to ICMR for their technical support.

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