PREVALENCE OF FUNGAL INFECTION IN CLINICALLY SUSPECTED CASES OF PULMONARY TUBERCULOSIS VISITING A TERTIARY CARE HOSPITAL

Rajani Shrestha, Niranjan Nayak, Dharm Raj Bhatt, Deependra Hamal, Shishir Gokhale

Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal

ABSTRACT

Pulmonary tuberculosis is the most important health concerns. Some fungi may acquire pathogenic potential in immunocompromised persons due to underlying diseases, use of prolonged antibiotics, chronic disease and malignancy. The presence of fungal pathogens in cases of pulmonary TB adds to the chronicity of the disease and being difficult to treat. This study aimed to evaluate tuberculosis (TB) status and coinfection of TB with pulmonary fungal infections. A total of 330 sputum samples were collected from suspected pulmonary TB and were examined using Ziehl-Neelsen (Z-N) staining method as per revised national tuberculosis control program (RNTCP) guidelines and GeneXpert assay procedure adopted, was in accordance with the WHO recommended guidelines. Those sputum samples were also processed for fungal culture. In case of any growth, this was identified by gram staining or by lactophenol cotton blue wet mount preparation and slide culture technique, if needed. A total of 29 (8.8%) samples out of 330 yielded tuberculosis by GeneXpert assay. Maximum positivity was noted among age group 31-45 years (15.5%). In the present study, GeneXpert positivity for the *Mycobacterium tuberculoris* (MTB) detected rate remained to be 8.8% (29/330) detected as against smear positivity in only 5.4% (18/330) (P value: 0.001). Out of 18, Z-N smear positive samples, maximum i.e. 17, which had yielded either high or medium detected of TB bacilli in the GeneXpert assay. Whereas, out the rest 12 GeneXpert positive (low and very low) samples, only one sample showed acid fast bacilli in the smear. A significant correlation was found between GeneXpert and smear positivity ($p<0.001$). Overall, 90 (27.7%) *Candida* spp. were isolated. Interestingly, 7 of these 90 *Candida* positive samples were found to positive to MTB by GeneXpert test, accounting a prevalence rate of 24.1% (7/29) of *Candida* coinfection among TB cases. Tuberculosis remains a global threat despite effort to eradicate the disease and TB co-infection with *Candida* spp. may complicate infection and treatment. In this present study, although the prevalence rates of all the coinfections were low and statistically not significant. Being chronic in nature and with confusing clinical and radiological findings, these fungal infections are misdiagnosed as reactivation of tuberculosis. Screening for TB should be conducted to diagnose early and treat these opportunistic infections and decrease mortality and morbidity rates associated with fungal coinfection in tuberculosis patients.

KEYWORDS

Candida, tuberculosis, GeneXpert

***Received on**: August 07, 2023*
***Accepted for publication**: September 06, 2023*

CORRESPONDING AUTHOR

Dr. Rajani Shrestha
Assistant Professor
Department of Microbiology
Manipal College of Medical Sciences,
Fulbari, Pokhara, Nepal
Email: rajani_sth7@hotmail.com
Orcid No: https://orcid.org/0000-0001-9499-7478
DOI: https://doi.org/10.3126/nmcj.v25i4.60871

INTRODUCTION

Pulmonary tuberculosis is a major cause of mortality attributed to the *Mycobacterium tuberculosis* (MTB) complex globally, tuberculosis (TB) is placed amongst the top 10 causes of death worldwide.[1] More than 95% of pulmonary tuberculosis cases have been reported from developing countries, particularly from Asia, Africa, the Middle East and Latin America which have limited diagnostic and therapeutic facilities.[2,3] Pulmonary tuberculosis is one of the most important health concerns. There are many risk factors such as immigration, family history of close contact with TB patients, social status, age, poverty, male gender, HIV infection, smoking, asthma and homelessness which had significant role in the risk of developing tuberculosis.[4]

Pulmonary fungal infection (pulmonary mycosis) is an infectious disease of the lungs caused by fungi. The infection develops after the colonization of the lungs by fungi or their spores through inhalation, or reactivation of latent infection, or via hematogenous dissemination. Filamentous saprophytic fungi have a wide distribution in nature, and their spore is abundant in the air and the transmission of infection occurs always through inhalation of spores.[5]

The extensive use of antibiotics and steroids has recently caused widespread fungal pulmonary infections. Some factors such as immunodeficiency, chronic diseases, malignancy were involved in worsening the condition.[6] In many instances, missed fungal pulmonary infection due to lack of specific clinical manifestations caused a high rate of morbidity and mortality.[7] The prevalence of opportunistic fungal infections which normally are incapable of causing disease in healthy persons has drastically increased in the recent past. These opportunistic fungi acquire pathogenic potential in persons who have compromised immunity due to underlying diseases, increased consumption of broad-spectrum antibiotics, or in those who harbour pulmonary tuberculosis. The fungal coinfection adds to the morbidity of pulmonary tuberculosis cases and becomes difficult to treat.[8]

Candida albicans is the most prevalent fungal pathogen that causes infections, including mild mucocutaneous infection to invasive forms affecting multiple organs, Severe *Candida* infections occur in immunosuppressive patients.[9] A prevalence of 15-40% has been reported in different studies on pulmonary tuberculosis coinfection with *Candida*.[10,11] Pulmonary fungal infections have clinical and radiological characteristics similar to tuberculosis which may easily be misdiagnosed as tuberculosis. Thus, in numerous cases of fungal pulmonary mycoses due to lack of specific clinical manifestations there be high rate of morbidity and mortality in patients initially suspected and treated for TB. Moreover, the presence of fungal pathogens in cases of pulmonary TB adds to the chronicity of the disease. This study aimed to TB status and coinfection of TB with pulmonary fungal infections in patients visiting Manipal Teaching Hospital in Pokhara, Nepal.

MATERIALS AND METHODS

This was an analytical observational study conducted between Aug 2022 to Feb 2023 in the Department of Microbiology, Manipal Teaching Hospital in Western Nepal, after obtaining approval from the Institutional Review Committee (IRC) (MCOMS/IRC/472). Patients clinically suspected to have pulmonary TB with symptoms such as productive cough for more than 2 weeks, accompanied by other respiratory symptoms such as shortness of breath, chest pains, hemoptysis and/or constitutional symptoms (loss of appetite, weight loss, fever, night sweats, and fatigue included in this study.[12]

A total of 330 samples were collected by consecutive sampling technique. The sample size was derived using formula sample size $n = z^2P(1-P)/d^2$, where n = sample size, z = level of confidence (1.96), p = expected prevalence, here in this study p = 30% 11,[13] d = precision (0.05) and minimum sample size obtained was 330. Sputum samples mixed with blood, food particles, not of enough volume were not processed. All the suspected sputum samples brought to microbiology laboratory for Z-N staining, GeneXpert and for culture were included while patient without clinical symptoms of pulmonary TB and patient receiving any antifungal agents were excluded.

Morning sputum specimens were collected from the patients after proper instructions so as to get ideal sample in a falcon tube of 50 ml capacity. At least 5 ml of sputum samples were collected from each patient. In case of in-patients who were unable to provide samples, sputum production was induced by nebulization with hypertonic saline.[14] All samples were sent to the Department of Microbiology, Manipal Teaching Hospital without delay.

A direct smear was prepared on a clean, dry and grease free glass slide using a clean disposable

wooden applicator stick. The smear was air dried, heat fixed and stained with Z-N staining method as per the revised national tuberculosis control program (RNTCP) guidelines.[15] Acid fast bacilli were seen as bright red/pink rods against blue background.

GeneXpert assay procedure adopted, was in accordance with the WHO recommended guidelines.[16] About 3 ml of the specimen was mixed with twice its volume of sample reagent. The mixture was then vortexed and incubated at room temperature for 10 minutes. Thereafter it was again vortexed and incubated for another 5 minutes. About 2 ml of this processed sample was then added to GeneXpert cartridge which was then loaded in the machine. The results were finally interpreted by the GeneXpert system based on fluorescent symbols which was displayed on the system monitor after about two hours.

The data were collected, entered and analysed using SPSS-17. Categorical variables were calculated as percentages. Chi-square test was used to compare two groups. All p values <0.05 were considered as statistically significant.

For fungal isolation, sputum specimens were inoculated onto two sets of Sabouraud's dextrose agar (SDA) with chloramphenicol, one incubated at 25°C and the other at 37°C. The SDA tubes were examined every day during the first week, and thereafter every alternate day up to a maximum period of 3 weeks. In case of any growth, this was identified by gram staining or by lactophenol cotton blue wet mount preparation and slide culture technique, if needed.[17] Statistical analyses of data were carried out by applying chi-square test.

RESULTS

This study aimed to evaluate TB status and co-infection of TB with pulmonary fungal infections in patients visiting Manipal Teaching Hospital. Table-1 depicts the positivity rates of pulmonary TB as tested by GeneXpert assay. A total of 29 (8.8%) samples out of 330 yielded *M. tuberculosis* by GeneXpert assay. Maximum positivity was noted among subjects belonging to of age group 31-45 years (15.5%) followed by those above 60 years (8.1%). Contrary to this, Z-N smear positivity was found in 18 (5.4%) samples only (Table 2).

Table 2 depicts the relationship between GeneXpert positivity and Z-N smear positivity. Out of 29 GeneXpert positive samples, 17 had

Table 1: Distribution of tuberculosis cases from clinically suspected TB according to age group

Age Group	Tuberculosis		TOTAL
	MTB Not detected	MTB detected	
1-15 year	2	0 (0)	2
16-30 year	30	3 (10%)	33
31-45 year	38	7 (15.5%)	45
46-60 year	39	2 (4.8)	41
More than 60 year	192	17 (8.1)	209
Total	**301**	**29 (8.78%)**	**330**

Table No 2: Density of M tuberculosis (MTB) detected by GeneXpert as compared to Z-N smear positivity

Density of MTB in GeneXpert	ZN Staining		
	Acid Fast Bacilli not seen	Acid Fast Bacilli seen	Total
High	0	3	3
Medium	0	14	14
Low	4	1	5
Very Low	7	0	7
MTB Not detected	301	0	301
Total	**312**	**18**	**330**

(P value significant< 0.001)

Table 3: Correlation of GeneXpert and Z-N staining positivity				
GeneXpert	**Z-N positive**	**Z-N negative**	**Total**	
Positive (29)	18*	11	29	
Negative (301)	0*	301	301	*p <0.001
Total (330)	**18**	**312**	**330**	

P value significant

Table No 4: Distribution of *Candida* spp. Isolated from patient of different age groups.			
Age Group	**Isolates of *Candida* spp.**		
	Not isolated	**Isolated**	**Total**
1-15 year	2	0 (00)	2
16-30 year	30	3 (10)	33
31-45 year	38	7 (15.5)	45
46-60 year	33	7 (21.2)	41
More than 60 year	136	73 (34.9)	209
Total	**240**	**90 (27.27)**	**330**

Table 5: Prevalence of TB and TB *Candida* co-infection (n=330)		
positive TB cases n (%)	**Positive *candida* cases n (%)**	**Positive TB and *candida* coinfection n (%)**
29 (8.78%)	90 (27.27%)	7 (24.13%)

yielded high and medium bacillary density and 12 yielded low and very low bacillary density. Whereas all 17 specimens showing high and medium grade were also smear positive; only one out of 12 low to very low GeneXpert positive sample was found to be positive. The above observation, undoubtedly signified the superiority of GeneXpert assay over microscopy. GeneXpert could detect *M. tuberculosis* from number of samples that were negative by the smear examination.

Correlation of positivity between GeneXpert and Z-N smear test was also analyzed and a significant correlation was found when a comparison was made between GeneXpert positivity and smear positivity (Table 3). As shown in Table 3, GeneXpert detected *M. tuberculosis* in all the 18 smear positive samples. In contrast to that, none out of the 301smear negative were GeneXpert positive. This difference was found to be statistically significant (p<0.001).

Of the all the samples were subjected to fungal culture, *Candida* spp. were isolated from 90 (27.7%; 90/330) specimens (P value: 0.001) (Table 4). Interestingly, 7 of these 90 *Candida* positive sputum samples were also positive for *M. tuberculosis* by GeneXpert test, accounting a prevalence rate of 24.1% (7/29) *Candida* co-infection among confirmed TB cases (Table 5).

DISCUSSION

Pulmonary tuberculosis (PTB) is a serious global health problem as well as a difficult disease to treat. The prevalence of opportunistic fungal infections has recently increased, which normally are incapable of causing disease in healthy persons.[8] These might become pathogenic in persons who have diminished immunity due to underlying diseases, increased consumption of broad-spectrum antibiotics, or who harbour pulmonary tuberculosis. In the present study, total of 330 sputum samples were obtained from clinically suspected PTB patients. Out of these, 167 samples were obtained from females whereas 163 samples were obtained from male patients.

All 330 sputum samples obtained from clinically suspected PTB patients were examined for both *M. tuberculosis* and *Candida* spp. Out of these 8.8% were positive for *M. tuberculosis* and 27.8% were positive for *Candida* spp.. Male patients were more affected by PTB (11.6%) as compared to females (6.0%). Similarly, male patients were affected more with candidiasis (29.4%) compared with their female counterparts. This was in agreement with the reports of WHO indicating male gender as a predisposing factor for tuberculosis infection.[2] This may possibly be related to higher environmental exposure of males than females to bacterial and fungal pathogens. Sani Fatima *et al*[19] also noticed that the distribution of fungal isolates varied with respect to the gender of the patients; males 63.5% and females 36.5%. Bansod *et al*[13] observed almost similar findings (males 62.5% and females 37.5%). The relatively higher colonization rates in males could be due to their outdoor activities during which they inhale fungal conidia (spores).

PTB positivity was more commonly seen in age group 31-45 years (15.5%) followed by age group of more than 60 years (8.1%). These facts may be due to the reason of increased exposure to the external environment and surroundings in males in that age group in addition to higher incidence of smoking and greater access to healthcare facilities in developing countries.[20]

Of the total sputum samples subjected to fungal culture, 90/330 (27.3%) were positive for *Candida* spp. Majority of culture positivity was seen in elderly patients (>60 year): 34.9 % followed by 46-60 year (21.2%) (Table 4). Elderly people were more vulnerable to fungal infections due to the decline and variable changes in the physiologic functions and also could be due to less production of microbiocidal peptide and protein in the oral cavity as well as lack of lysozyme with antimicrobial activity.[21,22] Therefore, age group is also one of the risk factors for pulmonary candida infection mainly due to diminishing immunity.

In this study, the prevalence of PTB was 8.7%, the prevalence of pulmonary fungal infection was 27.3% and the prevalence of TB *Candida* co-infection were 24.2% (Table 5) Among the *Candida*, *C. albicans* were the commonest among clinically suspected PTB patients. Fatima *et al*[19] reported the prevalence of PTB in 19.9%, pulmonary mycoses in 74.0%, and the prevalence of PTB-fungal pathogen coinfection in 6%. On the other hand, PTB fungal coinfection in the range of 18-40% was reported by other investigators.[21,22] The reason for increased fungal prevalence could be due to immunosuppression due to tuberculosis and the prolonged use of anti-TB drugs, which could promote the overgrowth of the fungi flora, and in turn, aggravate the course of the underlying pathology in the lung. On the contrary, Amiri *et al*[3] reported *Aspergillus* spp. as most predominant fungi, followed by *C. albicans* in pulmonary TB cases. Various *Candida* spp. have long been associated with pulmonary TB and have assumed the role of emerging pathogens in TB patients.[21] PTB might impair the host's immune system and increase the risk of invasive candidiasis in those individuals.[9]

Van Tongren *et al*[21] reported a case of co-infection due to *M. tuberculosis* and *Cryptococcus gatti* and suggested that infection with TB predisposed to infection with cryptococcus. In another study, Bansod *et al*[13] from India reported severe opportunistic fungal infections in PTB patients, advocating an immunocompromised state in such patients which facilitated fungal opportunists to colonize and invade in the lungs. Muni *et al*[23] studied a series of 200 cases of PTB and found that prevalence of TB fungal co-infection rate was low. However, they were of the view that TB fungus co-infection was a state of pathogenic synergism between both the conditions. Quite frequently pulmonary fungal infections in preexisting PTB are often misdiagnosed as reactivation of tuberculosis and hence underlying fungal infections goes unnoticed and untreated. In the above context, a more alarming situation was documented by Kali *et al*[11] from Pondicherry, India where majority of the patients with PTB fungus coinfection, had persistent pulmonary symptoms inspite of therapy. This happened because of co-infection with non albicans *Candida* spp. which inhererity resistant to many antifungal agents. The reported prevalence of fungal infection in PTB cases ranged between 12.7 and 36% the most common fungus being *C. albicans*.[11,22-25]

In the present study, GeneXpert positivity for the MTB was 8.8% against smear positivity in 5.4% of the samples. These findings were comparable to the findings of Mechal *et al*[23] in the context of high yield of MTB by GeneXpert assay as compared to the smear test. Munir *et al.* have also reported high detection rate of GeneXpert (77.4%) compared to smear positivity in (67.5%). GeneXpert being a molecular tool it is definitely superior to the smear microscopy. In addition, this study also highlighted that samples reported negative in smear examination could be detected by GeneXpert method. Similar observation was made by Umair *et al*,[23] where out of total 50 GeneXpert positive samples, Z-N staining was positive only in 30 samples. Despite the low positivity of Z-N smear as shown in our study, this technique cannot be totally ignored. Besides being a rapid and user-friendly tool, its results were found to be in good agreement with the density of mycobacterial yield detected by GeneXpert. However, the present study was done in a single centre with limited sample size and limited data, this cannot be generalized.

As the PTB remains a global threat, there is a high chance of *Candida*-TB co-infection causing wide range of clinical spectrum and chronicity complicating the situation; confusing clinical and radiological findings. In this present study, although the prevalence rates of all the coinfections were low and statistically not significant, the presence of these infectious agents in TB patients poses a greater risk. Hence, the routine screening for TB should be conducted to diagnose early and treat these opportunistic infections and decrease mortality and morbidity rates associated with fungal coinfection in tuberculosis patients.

Conflict of interest: None
Source of research fund: None

REFERENCES

1. Society AT. Diagnostic standards and classification of tuberculosis in adults andchildren. *Am J Respir Crit Care Med* 2000; 161: 1376-95.
2. WHO. Global tuberculosis report. Geneva: World Health Organization; 2013.
3. Amiri MJ, Karami P, Chichaklu AH *et al*. Identificationand isolation of *Mycobacterium tuberculosis* from Iranian patients with recurrent TB using different staining methods. *J Res Med Dent Sci* 2018; 6: 409-14.
4. Babamahmoodi F, Alikhani A, Yazdani Charati J *et al*. Clinical epidemiology and paraclinical findings in tuberculosis patients in north of Iran. *BioMed Res Int'l* 2015; 2015: 381572.
5. Hedayati MT, Mayahi S, Denning DW. A study on *Aspergillus* species in houses of asthmatic patients from Sari City, Iran and a brief review of the health effects of exposure to indoor Aspergillus. *Environ Monit Assess* 2010; 168: 481-7.
6. Osman NM, Gomaa AA, Sayed NM. Microarray detection of fungal infection in pulmonary tuberculosis. *Egypt J Chest Dis* 2013; 62: 151-7.
7. Pfaller M, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; 20: 133- 63.
8. Köhler JR, Casadevall A, Perfect J. The spectrum of fungi that infects humans. *Cold Spring Harb Perspect Med* 2015; 5: a019273.
9. Chen X-H, Gao y-C, Zhang Y, Tang z-H, YU Y-S, Zang G-Q. Tuberculosis infection might increase the risk of invasive candidiasis in an immunocompetent patient. *Rev Inst Med Trop Sao Paulo* 2015; 57: 273-5.
10. Naz SA, Tariq P. A study of the trend in prevalence of opportunistic candidal co-infections among patients of pulmonary tuberculosis. *Pak J Bot* 2004; 36: 857-62.
11. Kali A, Charles MP, Noyal MJ, Sivaraman U, Kumar S, Easow JM. Prevalence of *Candida* co-infection in patients with pulmonary tuberculosis. *Australas Med J* 2013; 6: 387-91.
12. Treatment of Tuberculosis: Guidelines. 4th edition. Geneva: World Health Organization; 2010. 2, Case definitions. Available from: https://www.ncbi.nlm.nih.gov/books/NBK138741/ https://apps.who.int/iris/handle/10665/44165
13. Bansod S, Rai M. Emerging of mycotic infection in patients infected with *Mycobacterium tuberculosis. World J Med Sci* 2008; 3: 74-8.
14. WHO. XpertMTB/RIF implementation manual: technical and operational "how to" practical consideration. WHO Press, France: GPS Publishing, 2014.
15. Tostmann A, Kik SV, Kalisvaart NA *et al*. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. *Clin Infect Dis* 2008; 47: 1135-42.
16. Agarwal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with Z-N stain and culture in samples of suspected pulmonary tuberculosis. *J Clin Diagn Res* 2016; 10: 9-12.
17. Chander J. Textbook of Medical Mycology (3rd edition). Mehta Publishers, New Delhi. India 2012.
18. Ndukwu C, Mbakwem-Aniebo C, Frank-Peterside N. Prevalence of *Candida* co-infections among patients with pulmonary tuberculosis in Emuoha, Rivers State, Nigeria. *IOSR J Pharm Biol Sci* 2016; 5: 60-3.
19. Sani FM, Uba A, Tahir F *et al*. Spectrum of pulmonary fungal pathogens, associated risk factors, and anti-fungal susceptibil- ity pattern among persons with presumptive tuberculosis at Gombe, Nigeria. *Int'l J Mycobacteriol* 2020; 9: 144-9.
20. Miller PB, Zalwango S, Galiwango R *et al*. Association between tuberculosis in men and social network structure in Kampala, Uganda. *BMC Infect Dis* 2021; 21: 1023. DOI: 10.1186/s12879-021-06475-z
21. Van Tongreen L, Shaipanich T, Flectharm JA. Co-infection with *Cryptococcus gatti* and *Mycobacterium tuberculosis* in an otherwise healthy 18 yr old women. *Case Report J* 2011; 18: 162-3.
22. Chinedum OK, Emwiomwan A, Ifeanyi OE, Babayi A. Comparative analysis of Ziehl-Neelsen and GeneXpert techniques for the diagnosis of tuberculosis in human immunodeficiency virus positive patients in Benin City. *Ann Clin Lab Res* 2017; 5: 1-6.
23. Umair M, Siddiqui S, Farooq M. Diagnostic accuracy of sputum microscopy in comparison with GeneXpert in pulmonary tuberculosis. *Cureus* 2020; 12: e11383.