Research Article

Co-Infection of Different Mycological Profiles in Active Pediatric Tuberculosis Patients

Shyam Sundar Bera*, Akash Das, Rupa Dasgupta, Tarun kumar Pathak, Devnil Pathak, Barnali Das, Rajdeep Paul, Debabrata Das

Department of Microbiology, North Bengal Medical College, West Bengal, India

Corresponding author:
Shyam Sundar Bera, Department of Microbiology, North Bengal Medical College, West Bengal, India; E-mail: Shyampathmicrobio16@gmail.com

Received: 03-Oct-2022, Manuscript No. amhsr-22-72512;
Editor assigned: 07-Oct-2022, Pre QC No. amhsr-22-72512(PQ);
Reviewed: 21-Oct-2022, QC No. amhsr-22-72512;
Revised: 28-Oct-2022, Manuscript No: amhsr-22-72512(R);
Published: 04-Nov-2022, DOI: 10.54608.annalsmedical.2022.73

Abstract

Background: Fungal elements can cause rigorous difficulty; generally in pediatric sufferers that have stumpy immunity to prolonged treatment of pulmonary tuberculosis. Many clinicians some time disregard fungal pulmonary infection, but it has a specific clinical manifestation for treatment purpose so that fungal pathogen can cause severe complication in treatment process in MDR tuberculosis.

Aim: The aim was to identify different overlapping mycological coinfection during the treatment process of a pediatric pulmonary tuberculosis patients and know the prevalence rate.

Method: The present study was conducted among 133 pediatric pulmonary tuberculosis patients with a subdivided groups of MTB and MTB-Rif and two specific age groups wise from Feb, 2020 to Oct, 2022 at Debra Super specialty Hospital, WB. Morning sputum samples were collected in a wide mouth sterile containers and then confirmed tuberculosis by TRUE-NAT (comprehensive assay for screening and confirmation of MTB and MTB-Rif). Then after confirmation of tuberculosis, during the treatment time, another repeated BAL (Bronchio Alveolar Lavage) samples were collected for presumptive diagnosis of fungal pathogen by using the preparation of KOH, gram stain, SDA culture and LPCB mount.

Results: *Aspergillus niger* showed high percentage in pulmonary tuberculosis of pediatric patients than other fungal elements for both MTB and MTB-Rif patients. 18.4% of *A.niger* saw 11-15 years of age group and 13.1% seen in between 4-10 years of age group in male patients. Similarly, 7.8% in 4-10 years age group and 10.5% in 11-15 years of age group found *A. niger* in female patients with MTB pediatric patients. Only 33.3% *A.niger* was seen in females in the particular age group of 4-10 years in MTB patients and 66.6% was seen in male patients in MTB-Rif patients in particular age group of 11-15 years. Aspergillus fumigates was seen 7.8% in males and 5.2% in females in the age group of 11-15 years in MTB patients but such fungal agent was not seen in MTB-Rif patients. Maximum percentage of A. flavus was seen in 5.2% and *H. capsulatum* 2.6% in different two-separated age groups. Cryptococcus neoforman was showed 5.2% in MTB patients.

Conclusion: Mycological agents can cause harmful effecst on MTB and MTB-Rif treating patients. In that situation, only treatment of pulmonary tuberculosis is not a prior step for improvement MTB or MTB-Rif. Clinicians should take instant action against fungal pathogens causing harm in an active pulmonary pediatric tuberculosis patients and without clinician advice should not take any medicine; otherwise tubercle bacilli can cause genetic changes, due to the effect Multiple Drug Resistant Tuberculosis (MDR-TB) or Extensively Drug Resistant Tuberculosis (XDR-TB). Therefore, this presents a need for routine diagnosis of pulmonary mycosis among TB suspects and set-up of an antimicrobial profile for pulmonary fungal isolates to support clinical management of these cases.

Keywords: Multiple Drug Resistant Tuberculosis (MDR-TB); Extensively Drug Resistant Tuberculosis (XDR-TB); True-NAT; Pulmonary Tuberculosis (PTB)

Introduction

The burden of pediatric fungal respiratory tract infection is obscure; the frequency of infection has been increasing in the last few decades. Fungal pulmonary infection has been emerging recently due to widely used broad spectrum antibiotics and steroids[1]. In Asia, the annual incidence of TB is very high, reaching hundreds of cases per 100,000 people For instance, the role of pulmonary fungal infections caused by pulmonary fungal pathogens has been highlighted recently and the similarity in clinical and radiological characteristics with TB further complicates the diagnosis and management of such pulmonary infections[2,3]. Indeed, pulmonary mycoses can mimic and easily be misdiagnosed as TB and vice versa. In this context, fungi have over time gained attention for their recent emerging medical importance worldwide[4]. Over the past 30 years now, fungi have transitioned into key an etiological agents for difficult to manage infections, killing at least one million people annually; and yet remain among the neglected

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

How to Cite this Article: Bera SS, et al. Co-Infection of Different Mycological Profiles in Active Pediatric Tuberculosis Patients. Ann Med Health Sci Res. 2022;12: 373-377.

© 2022 Annals of Medical and Health Sciences Research

diseases globally[5]. Serious invasive fungal infections occur in immune-compromised patients such as HIV/AIDS, cancer and transplantation patients among others, in many of whom they complicate and worsen the disease. PTB is principally a disease of poverty, with 95% of cases and 98% of deaths occurring in developing countries. Ethiopia stands 10th among the 30 high TB burden countries with an estimated incidence rate of 151/100,000. The high rate of co-infection of pulmonary fungal infection with PTB further compounded the burden of PTB in these countries as the association of the two infections is responsible for a high rate of morbidity and mortality. Therefore, proper diagnosis of fungal pathogen especially in PTB patients is critical. The percentage of mycotic infections increase in pulmonary tuberculosis patients. Mainly four types of fungi, *i.e. Aspergillus niger*, *A. fumigatus*, *Histoplasma capsulatum* and *cryptococcus neoformans* were recorded, which causes severe infection in lungs in patients suffering from pulmonary tuberculosis. Many physicians missed fungal pulmonary infection

because it does not show specific clinical manifestations and usually hindered by other diseases and cause high rates of morbidity and mortality. There is an increasing awareness amongst clinicians and microbiologists pertaining to importance of infection caused by opportunistic fungi. Therefore there is an acute need for proper diagnosis of the opportunistic fungal pathogen especially in tuberculosis patients. The conventional identification of pathogenic fungi based on phenotypic features and physiological tests is time-consuming and, therefore, often imperfect for the early initiation of an antifungal therapy.

Material and Method

Study area and design

The present study is a hospital-based, cross-sectional study conducted at Debra super speciality Hospital, WB from Feb, 2020 to Oct, 2022. Laboratory test was done at Debra Thana Sahid Khudiram Smriti Mahavidyalaya,in the Department of BMLT (Laboratory Science).

Population

The source of population is those people seeking health service at NTEP (National Tuberculosis Elemination Programme) cell of Debra super speciality Hospital,WB. Few patients referred from different subcentre including this area.

Sample size

Sputum samples were collected from 133 clinically PTB confirmed pediatric patients referred to TB clinic for continued treatment process.

Inclusion criteria of study participants

Patients with confirmed diagnosed of PTB who visited the TB unit who required MTB True-NAT (molbiol) diagnostic test were included. Signed informed consent was sought from each study participant prior to sputum sample collection. As for children, written informed assent was obtained from their care givers.

Exclusion criteria

TB suspects who failed to provide sputum samples and whose request forms lacked demographics were excluded from this study.

Specimen collection

First morning sputum sample for MTB True-NAT (molbiol) and fungal culture was collected aseptically from study participants in a sterile dry wide-necked, leak-proof universal container from each study participant.

Transport and storage of samples

Samples were transported in courier boxes from the point of collection to the laboratory. Samples which were not processed within the same day of collection were stored in a refrigerator at 4°C–8°C awaiting processing.

Specimen processing

Collected sputum samples were processed at Department of BMLT in Mycology Laboratory Unit under Debra Thana Sahid Khudiram Smriti Mahavidyalaya.

True-NAT (molbiol) MTB/Rifampicin (RIF) assay

1 ml sputum sample was added to liquid Lysesd buffer (1:5) ratio and wait for 10 min up to liquefaction of mucoid part of sputum, then 2 ml of liquefied solution transfer into cartage for nucleic acid amplification,it will takes 20 min. after amplification, take 5 µl DNA of sputum sample mixed with the design primer (supplied with MTB/RIF chip) and run on the amplifier machine for 60 min. result showed MTB/MTB-Rif detected or Not-Detected.

Identification

Potassium hydroxide (KOH) mounts: A drop of 10% KOH was placed on a clean glass slide using Pasteur pipette. A small portion of sputum was added into the KOH drop using a sterile wire loop and mixed well. The coverslip was placed on top of this mixture and the preparation was placed in a moist chamber and kept at room temperature for 30 min. The preparation was examined under low power microscope objectives for the presence or absence of fungal elements.

Gram stain: Gram stain smear was made from the sputum sample to test for the Gram reaction of fungi and the size, shape and arrangement of fungal elements. For Gram positive yeast-like cells, mucopurulent absence or presence of pseudo hyphae was recorded.

Fungal culture: Sabouraud Dextrose Agar (SDA) containing antibiotic chloramphenicol and gentamicin was used to culture sputum samples. The specimens were streaked onto the medium in the Universal bottles with a sterile inoculating loop in order to obtain isolated colonies. The preparations were then incubated at 25°C–30°C in an inverted position (agar side up). Cultures were examined at least weekly for fungal growth and held for 4 weeks before being reported as negative. After sufficient incubation, considering colony morphology, texture, rate of

growth, surface of the colony and pigmentation on the surface and reverse of the colony on SDA tubes were recorded. The significant fungal isolates on culture were identified to the species level, using standard mycological procedures.

Lactophenol cotton blue staining: Lactophenol cotton blue was used for microscopic identification and characterisation of fruiting bodies such AS conidia, sporangia, rhizoids and hypha or mycelia of cultivated fungi on SDA. A drop of lactophenol cotton blue stain was placed on a clean grease-free glass slide. A small fragment of cottony, woolly or powdery colony was picked from the midpoint of the culture using a sterile straight wire and placed on a clean glass slide for the staining process. A clean coverslip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation examined using X10 and X40 objectives of the microscope. Fungal element features such as microconidia, macroconidia, chlamydospores and hyphae with spiral, pertinate and antler-like structures were investigated. These features seen on the stained slide were compared with established characteristic fungal features using mycology atlases.

Safety and environment

All biological specimens, including used cartridges, capable of transmitting infectious agents were treated with universal precautions. All laboratory procedures were done in a level 2 TB laboratory. Personal protective equipment such as disposable gloves, laboratory coats were used when handling specimens and reagents. Washing of hands was done thoroughly after handling specimens and test reagents. Disposing of used True-NAT MTB/RIF cartridges was done according to the country's safety guidelines for hazardous material.

Statistical analysis

Results were analyzed using Pearson's chi-square test, or Fisher's exact test. A value of $P<0.05$ was regarded as statistically not significant (SPSS 16.0, SPSS Inc. Chicago, IL, USA).

Results

Out of 133 positive pediatric tuberculosis patients, 90.9% (n-121) showed normal MTB patient and 9% (n-12) showed MTB-Rifampicin positive pulmonary tuberculosis patient. After doing culture on that collected sputum sample from those patients found 31.4% (n-38) fungal growth from MTB pediatric patient and 25% (n-3) fungal growth seen in among MTB-Rif patient.

According to distribution of male and female showed 22.3% pediatric in between age group 4-10 years and 77.6% showed in between age group 11-15 years in MTB case, similarly 19.4% showed in female age group of 4-10 years and 80.5% seen in age group of 11-15 years in case of MTB pediatric patient. In MTB-Rif positive patients had seen 16.6% male and 8.3% female in 4-10 years of age group (Table 1). Similarly, in the age group of 11-15 years seen 50% male and 25% female patient in recent case study.

In recent study showed different fungal element in both MTB and MTB-Rif positive pediatric patients. As per distributionin between two age group of male and female patient showed four species of mycological pathogen in different percentage (Table 2). Highest number of fungal pathogen seen 49.8% *Aspergillus niger*, 20.8% *Aspergillus fumigates*,13% *Aspergillus flavus*,10.4% *Criptococcus neoformans*, 5.2% *Histoplasma capsulatum* in specially MTB patient.

Our study showed 39.2% fungal infection in pediatric MTB among male and 20.9% in female between 11-15 years of age group. Significant mycological growth also seen in age group of 4-10 years of age of 23.5% in male and 15.6% in female pediatric. Another analysis data was seen in Rifampicin resistant tuberculosis pediatric patient, here only 33.3% (n-1) *Aspergillus niger* found in age group of 4-10 years of female patient and 66.6% (n-2) seen in 11-15 years of age group among male patient. No other mycological profile seen among MTB-Rif patient.

Table 1: Distribution of two age group between MTB and MTB-Rif.

	MTB (121)		MTB-Rif (12)		P Value
	Fungal growth	No fungal growth	Fungal growth	No fungal growth	
	38 (31.4%)	83 (68.5%)	3 (25%)	9 (75%)	3.016 Non-significant
Age group wise distribution	Male (85)	Female (36)	Male (8)	Female (4)	
4-10 yrs	19 (22.3%)	7 (19.4%)	2 (16.6%)	1 (8.3%)	Significant Value ≤ 0.05
11-15 yrs	66 (77.6%)	29 (80.5)	6 (50%)	3 (25%)	

Table 2: Prevalence rate of fungal species between male and female among pediatric pulmonary tuberculosis.

Different Mycological agent	MTB (121)				MTB-Rif (12)			
	4-10 yrs		11-15 yrs		4-10 yrs		11-15 yrs	
	Male	Female	Male	Female	Male	Female	Male	Female
Aspergillus niger	5 (13.1%)	3 (7.8%)	7 (18.4%)	4 (10.5%)	0	1 (33.3%)	2 (66.6%)	0
Aspergillus fumigates	1 (2.6%)	2 (5.2%)	3 (7.8%)	2 (5.2%)	0	0	0	0
Aspergillus flavus	2 (5.2%)	0	2 (5.2%)	1 (2.6%)	0	0	0	0
Histoplasma capsulatum	0	1 (2.6%)	1(2.6%)	0	0	0	0	0
Criptococcus neoformans	1 (2.6%)	0	2 (5.2%)	1 (2.6%)	0	0	0	0

Note : P value-0.99; Showing not significant result; Significant Value ≤0.05

Discussion

As per protocol of National Tuberculosis Elimination Programme (NTEP) maximum 96.8% children are already vaccinated by BCG, although fungal infections remain a leading cause of infectious morbidity and mortality in heavily among tuberculosis patient.

Mycological growth cans severe cause for the collapsing lungs during treatment process of tuberculosis. Most of pulmonary tuberculosis patient are immunocompromise that a reason for opportunistic infection occurring functionally. In current study showed 31.4% (n-38/121) fungal growth in MTB patients and 25% MTB-Rif (n-3/12). All patients including study are getting treatment more than three month or more. Similarly, the study was done by Njovu, et al. at Southwestern Uganda; they showed 70.7% (80/113) total fungal growth from collected sputum samples[6].

As per distribution of two different age group 22.3% (n-19) were female and 19.4% (n-7) were male in 4-10 years of particular age group in MTB patients, otherwise 16.6% (n-2) male and 8.3% (n-1) female are MTB-Rif patients were included in our study.

Similarly another age group from 11-15 years included 77.6% (n-66) male and 80.5% (n-29) were in female patients in treated with MTB, in MTB-Rif treated 50% (n-6) was male and 25% (n-3) were female. In contrast, study was conducted by Shivam et al. in Burdwan, West Bengal, India[7], they showed among 758 patients, male was 577 (61%) and female was 181 (23%) and Mwaura et al. in Nairobi, Kenya, they showed among 172 patients, female was 69 (40.1%) and male was 103 (59.9%) respectively[8]. In most of study showed maximum case of TB patents are male, due to survival of life they have to go outside from home for their needy requirement, another cause must be unhygienic living style and food habit. In our recent case study with pediatric pulmonary tuberculosis can be unhealthy lifespan or may be close contact. Few cases are seen with family member treated with pulmonary tuberculosis, they are not maintaining proper precaution not to spread such diseases.

Due to squat immunity of those tubercle patients and also not maintaining proper protocol of guidelines, most of are affected in bacterial, viral, parasitic of fungal infection. In current study showed different mycological profile during treatment interval. Its may be reason of unhealthy system maintain or may be poor life style with protein energy malnutrition. Our current result showed 13.1% (n=5) in male and 7.8% (n=3) female of *Aspergillus niger* in 4-10 years of age group among MTB patients. Similarly, total 3 (1 male and 2 female) patients are found in 11-15 years of age group in MTB-Rif treated. This particular mycological agent causing more opportunistic infection in active tuberculosis patient, here we also showed in highest rate than other fungal agent. We also showed in another *Aspergillus* spp. in lower percentage like 20.8% (n=8) *Aspergillus fumigates*, 13% (n=5) *Aspergillus flavus* in our study. This finding in accordance with Amiri, et al. in Ghaemshahr City showed that among mycological co-infected 91 male patients. Of which *Candida albicans* 1 (6.25%), *Aspergillus niger* 1 (6.25%) and Aspergillus fumigatus 5 (31.25%)[9]. However, a much higher frequency has been reported Bitew, et al., Ethiopia, showed that among 562 isolates patients, where C. albicans 260 (52.7%). And the remaining 128 (18.6%) isolated mycelial fungi, where Aspergillus spp. 79 (61.7) in active tuberculosis patient in adults[10]. Those mycological spores can spread person to person with close contact cause infection in lungs and also producing black pigmentation, we observed in BAL sample collected from patients.

Another mycological findings we observed in our current study, most of case study very less number *Histoplasma capsulatum* and *Criptococcus neoformans* are reported, But we showed 5.2% (n=2) *Histoplasma capsulatum* and 10.4% (n=5) Criptococcus neoformans significantly in MTB patient in two different age group. Normally *Cryptococcus* spp. seen in older heritage where source of nitrogen compound originate *via* any route, it is the favorable condition to grow *Cryptococcus* spp. such findings cannot compare with another study because maximum case study associated with active tubercle adult patient. This finding is very similar to another mycological profile, Mathavi, et al. they showed that *Candida albicans* was the predominant species causing secondary infection in tuberculosis patient[10]. Out of 21 Candida isolates, 14 were identified as *C. albicans*

(66.7%), 2 were *C. tropicalis* (9.5%), 2 were *C. krusei* (9.5%) and C. parapsilosis was also isolated from 2 cases (9.5%). C. glabrata was isolated from one sample (4.8%).

Such other mycological profile can see in long term study with area basic. Other mycelia spp. can grow in TB patient; these are common opportunistic fungal pathogen.

Conclusion

Pediatric pulmonary tuberculosis can be a devastative form in expectations, within several mycological opportunistic infections can be a leading serious problematic cause of treatment protocol. We found limited evidence of factors that influence epidemic and host's immunity of co-infection between TB and fungal disease in humans. Most of common fungal pathogen is Aspergillus spp. in both MTB and MTB-Rif patients. Pediatric pulmonary tuberculosis associated with fungal infection was shown to be risk factors for each other. Co-infection may inhibit the hosts immune system to a great extent. In addition, infection with fungal can alter the protective immune response to BCG vaccination against both MTB and MTB-Rif patient. Tuberculosis treatment protocol can be hampering without proper medication against opportunistic fungal pathogen, it may be fatal cause in pediatric patient treating pulmonary tubercle. In that sence, in active tuberculosis patients those are diagnose primarily and taking medicines as per guidelines of DOTS, they can expose frequency common mycological pathogen in during the treatment of the tuberculosis, because of there are immune compromised long persistence patients. They need to employ predictable microbiological tests along with clinical and radiological evidence since clinical manifestations and radiological pictures of PTB mimic that of pulmonary fungal infection. In that condition of patient during treatment of TB must follow clinician's guideline and not roaming outside with environmental exposure, it may lead to pulmonary mycological infection in Active Tuberculosis patient.

Acknowledgements

The authors would like to thank the Department of Medical Laboratory Science, Debra thana sahid khudiram smriti Mahavidyalaya and Debra super speciality Hospital for allowing the authors to use laboratory facilities for free. Special thanks to Prof Dr. Tarun Kumar Pathak, (MSVP,Bankura Sammilani Medical College,WB) inspiring me to continue the project work. The authors also would like to acknowledge study participants for their participation in the study. They are also very grateful to the medical laboratory technologists for their help during sample collection.

References

1. Fauci AS, Morens DM. The perpetual challenge of infectious diseases. N Engl J Med. 2012; 366: 454–461.
2. Nucci M, Marr KA. Emerging fungal diseases. Clin Infect Dis. 2005;41: 521–526.
3. MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global epidemiology of tuberculosis and progress toward achieving global targets-2017. MMWR Morb Mortal Wkly Rep. 2019; 68: 263–266.
4. Holt MR and Chan ED. Chronic cavitary infections other than tuberculosis. J Thorac Imaging. 2018; 33: 322–333.
5. Warnock DW. Trends in the epidemiology of invasive fungal infections. Nihon Ishinkin Gakkai Zasshi 2007; 48: 1–12.
6. Njovu IK, Musinguzi B, Mwesigye J, Kassaza K, Turigurwa J, et al. Status of pulmonary fungal pathogens among individuals with clinical features of pulmonary tuberculosis at Mbarara University Teaching Hospital in Southwestern Uganda. Ther Adv Infectious Dis. 2021;8: 1–11.
7. Shivam S, Saha I, Mondal TK, Misra RN, Dasgupta S, et al. Gender differentials in tuberculosis: An Experience from a Rural Tuberculosis Unit of Burdwan District, West Bengal, India. TAF Preventive Medicine Buletinl.2014;13:109-114.
8. Mwaura EN, Matiru V, Bii C. Mycological findings of sputum samples from pulmonary tuberculosis patients attending tb clinic in Nairobi, Kenya. Virology&Mycology.2013;2:119.
9. Amiri MRJ, Siami R, Khaledi A. Tuberculosis status and coinfection of pulmonary fungal infections in patients referred to reference laboratory of health centersghaemshahr city during 2007-2017. Ethiopian Journal of Health Science. 2018;28:683-690.
10. Mathavi S, Shankar R, Kavitha A, Sasikala G, Priyadharsini I. A study on prevalence of pulmonary candidiasis among tuberculosis patients and use of chromagar in identification of candida species. Journal of Drug Delivery & Therapeutics. 2014: 4;118-121. ,