Section: Miscellaneous

Original Research Article

A STUDY ON THE PREVALENCE OF PULMONARY PARAGONIMIASIS AND NOCARDIOSIS IN CLINICALLY SUSPECTED PULMONARY TUBERCULOSIS PATIENTS TESTED NEGATIVE BY DIRECT SPUTUM SMEAR EXAMINATION AND MOLECULAR METHOD

Prahllad Debnath[1], Pranab Bhaumik[2], Khuraijam Ranjana Devi[3], Thongam Nabakumar Singh[4], Sunanda Haorongbam[5], Subhrajit Bhattacharjee[2]

[1]MD Microbiology, Tripura Health Service, Agartala, Tripura, India.
[2]Post Graduate Trainee-III, Department of Microbiology, RIMS, Imphal, India
[3]Professor, Department of Microbiology, Head of Research and Development and Laboratory Director, Mother's Care Children Hospital and Research Center, Imphal, India
[4]Professor, Department of Microbiology, RIMS, Imphal, India.
[5]Professor and Head, Department of Respiratory Medicine, RIMS, Imphal, India

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Corresponding Author:
Dr. Prahllad Debnath,
Email: doc.prahllad@hotmail.com

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Abstract

Background: The last prevalence study on paragonimiasis in Manipur was conducted in 1993 in Imphal East, reporting a prevalence of 6.7%. No follow-up studies have been conducted since. For nocardiosis, limited studies exist both in India and globally. This study aimed to determine the prevalence of paragonimiasis and nocardiosis in clinically suspected tuberculosis (TB) patients who tested negative for Mycobacterium tuberculosis (MTB) by molecular methods (CBNAAT) and Ziehl-Neelsen (ZN) staining. **Materials and Methods:** This cross-sectional study was conducted at RIMS, a tertiary care hospital in Manipur, from September 2019 to October 2021. A total of 100 clinically suspected TB patients (61 males, 39 females) of all age groups were included. Samples were examined using Gram stain, ZN stain, Kinyoun stain, and wet mount. Cultures were performed on blood agar, nutrient agar, Lowenstein-Jensen (LJ) media, Sabouraud Dextrose Agar (SDA), and paraffin baiting technique. **Result:** Nocardia spp. was detected in one sample (1%) using direct smear and confirmed via culture. No ova of Paragonimus spp. were detected in the samples. The 1% prevalence of nocardiosis was consistent with findings from other studies in India and globally. The zero prevalence of paragonimiasis may be attributed to the change in food habits, awareness and small sample size. **Conclusion:** The study highlights a low prevalence of nocardiosis and an apparent absence of paragonimiasis among clinically suspected TB patients. Larger studies with more sensitive diagnostic techniques are necessary to accurately determine the prevalence of these infections in Manipur.

INTRODUCTION

Paragonimus spp., a member of the genus Trematoda and family Paragonimidae, is responsible for paragonimiasis, also known as Oriental lung fluke, pulmonary distomatosis, and benign endemic hemoptysis.[1] India's northeastern states, including Manipur, Nagaland, Arunachal Pradesh, and Assam, are endemic to paragonimiasis, a neglected tropical disease affecting approximately 1 million people annually, with common species being Paragonimus westermani and heterotremus.[2]

Human infection primarily results from the consumption of raw or undercooked, pickled, brine-soaked crabs, crayfish, and shrimps that harbor the infective form. The parasites utilize a wide variety of freshwater snails and crustaceans like crabs, crayfish, and shrimps as first and second intermediate hosts, respectively and they can remain inactive for many years inside the infected crabs.[3] On the other hand, Nocardia spp. is aerobic, gram-positive, filamentous, partially acid-fast, branched bacteria which were initially classified under fungi because they possessed true aerial hyphae, which were considered to be a fungal characteristic.[4] However, based on

their cell wall components such as their cell envelope lipid and peptidoglycan compositions, these microorganisms are now recognized as true bacteria.[5] The incidence of human disease has increased substantially in the last two decades in association with an increasing population of immunocompromised hosts and improved methods for detection and identification of Nocardia spp. in the clinical laboratory.[6] Nocardia spp. usually does infect immunocompromised patients, whereas it has also been reported from immunocompetent patients. Thus, irrespective of a patient's immunologic status the isolation of Nocardia spp. from the respiratory tract or other body sources should not be regarded as a contaminant or commensal organism. Patients with depressed cell-mediated immunity especially are at high risk for infection, including those with lymphoma, other selected malignancies, human immunodeficiency virus infection, and solid-organ or hematopoietic stem cell transplant and those receiving long-term treatment with steroids or other medications that suppress cell-mediated immunity.[7] Genus Nocardia, Gordonia, Mycobacterium, and Rhodococcus are phenotypically similar and belong to Family Actinomycetes. To differentiate between them, characteristics like colonial morphology properties, production of pigment, gram stain feature, acid fastness, partial acid fastness, and production of aerial hyphae are used. Recently, modern tools like matrix-assisted laser desorption/ionization (MALDI-TOF mass spectrometry) and PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) have been used for accurate identification of this organism.[8] As per the TB India report 2018, the estimated incidence of tuberculosis in India was 2.8 million approximately. In Manipur, out of the 2,805 tuberculosis patients that have been notified up till 2017, 36% were clinically diagnosed with tuberculosis in the year 2017 alone.[9]

Patients suffering from infections like paragonimiasis and nocardiosis usually complain of fever, cough, and hemoptysis which overlaps with signs and symptoms of tuberculosis including their radiological features. There have been cases in Manipur that were initially treated for tuberculosis but due to their unresponsiveness to anti-tubercular therapy, a reevaluation was undertaken where eventually a diagnosis of paragonimiasis was confirmed.[10]

Such diseases are usually misdiagnosed as tuberculosis which then leads to unnecessary exposure of patients to anti-tuberculosis drugs. In a state like Manipur, which is endemic to paragonimiasis, careful clinical evaluation and appropriate steps of diagnosis are important criteria for proper management of this disease. Keeping this in mind, the study aims to evaluate the prevalence of pulmonary paragonimiasis and nocardiosis among suspected pulmonary tuberculosis patients.

Aim: To determine the prevalence of pulmonary paragonimiasis and nocardiosis in clinically suspected pulmonary tuberculosis patients.

MATERIALS AND METHODS

Settings: The study is a multi-centre cross-sectional study conducted from September 2019 to October 2021 in the Mycobacteriology section, Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, India; Department of Respiratory and Chest Medicine RIMS, Imphal, India and The Intermediate Reference Laboratory, Research and Development Wing, Imphal West, India. The study was approved by the Medical Ethical Research Committee of the Institute.

Inclusion criteria:

The inclusion criteria are as follows

- Patients with suspected pulmonary tuberculosis.
- Negative results on direct sputum smear examination.
- Negative results on molecular testing using CBNAAT.
- North-East Indian population or North-East Indian ethnicity

Exclusion criteria:

The exclusion criteria are as follows:

- Patients already diagnosed with tuberculosis.
- Patients with inadequate samples or leaking containers.
- Patients with terminal illnesses like cancer, and chronic conditions like liver failure.
- Patients unwilling to provide consent.

Measurements: This cross-sectional study uses a sample size of 100 patients, calculated based on a 6.7% prevalence rate of paragonimiasis from Singh SK et al., using consecutive sampling. Independent variables include age, gender, residence, history of HIV, immunosuppressive therapy, and raw/undercooked crab intake, While the outcomes are the prevalence of paragonimiasis and nocardiosis. Data were collected through a structured questionnaire and analyzed using statistical methods. Laboratory tools include staining techniques, culture media, and CBNAAT for molecular diagnostics. Prevalence rates and associations between variables were evaluated using descriptive and inferential statistics.

Specimen Collection Procedures: Sputum samples were collected following standard procedures. Either one spot and one morning sample or two spot samples with at least a one-hour gap for patients unable to return. Other specimens included pleural fluid, bronchoalveolar lavage, and gastric aspirates (for children or non-sputum-producing patients). Sputum (2–5 ml, preferably blood-stained or muco-purulent) was collected in sterile 50 ml conical tubes after mouth rinsing and transported promptly. For children unable to produce sputum, stool samples were collected in clean, leak-proof containers, avoiding

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contamination, and transported to the lab within an hour.

Laboratory Examination and Identification Methods: Pulmonary specimens were examined for Paragonimus spp. eggs using direct wet mount, with muco-lysis by 3% NaOH and concentration by centrifugation. Stool samples underwent formalin-ether concentration and direct wet mount in saline and iodine. For nocardiosis, Gram stain, ZN stain, Kinyoun stain, and 10% potassium hydroxide wet mount were used, along with inoculation on paraffin bait technique, blood agar, LJ media, and Sabouraud dextrose agar, incubated at 37°C for 3–4 weeks. Isolates were identified using biochemical tests and microscopic examination. Paraffin baiting involved mixing specimens with a sterile broth and paraffin-coated glass rods.

Statistical analysis: Statistical analyses were conducted using the IBM SPSS version 21 statistical software package (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used for the analysis of age wise distribution, sex and location (district) wise distribution. Prevalence rates and associations between variables were evaluated using descriptive and inferential statistics. Continuous variables are presented as Means ± Standard deviation. C $P < 0.05$ was considered the statistically significant value.

RESULTS

In the present study, a total of 100 sputum samples, all negative for Mycobacterium tuberculosis by cartridge-based nucleic acid amplification test (CBNAAT) and Ziehl-Neelsen acid-fast staining, were. Of these, 61% (n=61) were from male participants, while 39% (n=39) were from females. Patients' ages ranged from 17 to 86 years. The largest proportion of samples were from the 40–49 years and ≥60 years age groups (22%, n=22), followed by 50–59 years (15%, n=15), 30–39 years (12%, n=12), and 20–29 years (4%, n=4). The smallest group was those aged <19 years (3%, n=3) [Figure 1]. The majority of patients were from the Imphal West district (47%, n=47), followed by Thoubal (14%, n=14) and Imphal East (13%, n=13). The fewest patients were from the Kakching district (1%, n=1) [Figure 2]. The majority of patients (66%) presented with cough as their primary complaint, followed by fever in 32% of cases. A smaller proportion of patients (2%) reported chest pain. These symptoms highlight the most common manifestations among the study population, with cough being the predominant symptom. These findings suggest that respiratory symptoms, particularly cough and fever, were the most prevalent among the study participants [Table 1].

None of the patients reported consuming undercooked or raw crab in the six months leading up to the onset of symptoms, suggesting that dietary factors may not have contributed to the infection in this cohort. Among the patients, three tested positive for HIV, indicating potential immunocompromised status. Additionally, one patient had a history of kidney transplant and was receiving immunosuppressive therapy, which may have influenced their susceptibility to infections. These factors highlight the importance of considering underlying health conditions in the context of infectious disease presentations [Table 2]. [Table 3] observed that Nocardia spp. was isolated from one patient, yielding a prevalence rate of 1%. Wet mount microscopy did not reveal any Paragonimus spp. ova, indicating the absence of this infection in the samples examined. The direct Gram stain and Kinyoun stain of the sample, demonstrating the presence of Gram-positive branching bacilli and branching acid-fast bacilli is shown in [Figure 3A and 3B] respectively. The Gram stain shows Gram-positive branching bacilli, while the Kinyoun stain highlights acid-fast branching bacilli. Both stains were captured at 100X magnification, emphasizing the characteristic branching morphology and acid-fastness, key features in identifying Nocardia species. These staining techniques help differentiate Nocardia from other microorganisms by enhancing the visibility and detection of the bacteria in clinical samples. [Figure 3A]. The Gram stain from Lowenstein-Jensen (LJ) media shows Gram-positive branching bacilli at 100X magnification, characteristic of Nocardia spp., aiding in its identification [Figure 4A]. Whereas the Kinyoun stain from paraffin baiting technique media reveals acid-fast non-branching bacilli at 100X magnification, demonstrating strong acid-fastness, a key feature of Nocardia spp., despite the absence of typical branching morphology [Figure 4B].

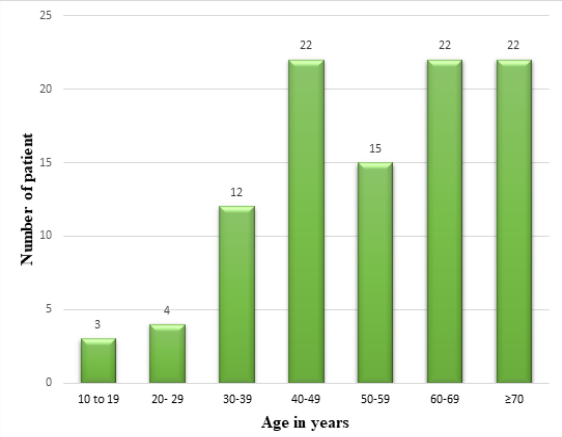

Figure 1: Age-wise distribution of patients (N=100).

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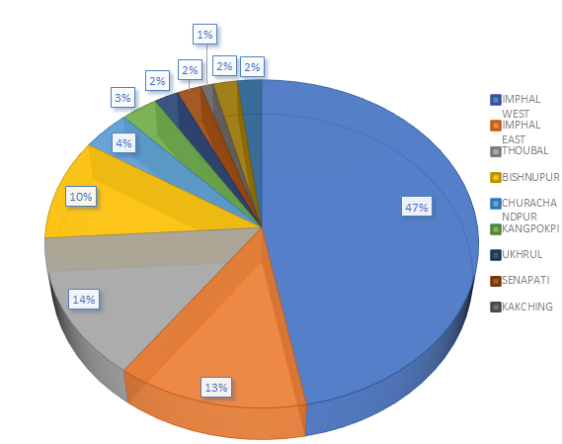

Figure 2: District-wise distribution of patients

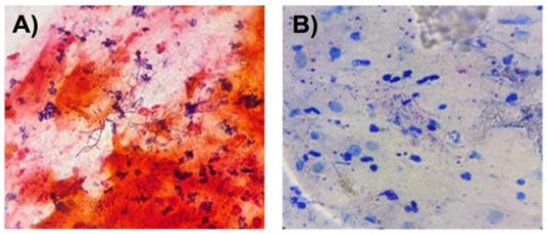

Figure 3: (A) Direct Gram stain of the sample, demonstrating the presence of Gram-positive branching bacilli. (B) Direct Kinyoun stain of the sample, revealing branching acid-fast bacilli.

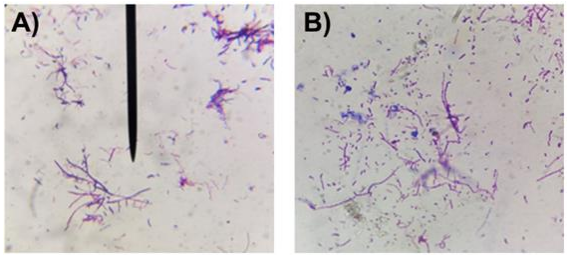

Figure 4: (A) Gram stain from LJ media showing gram positive branching bacilli (100X) (B) Kinyoun stain from paraffin baiting technique media showing acid-fast non-branching bacilli (100X)

Table 1: Distribution of patients according to symptoms (N=100).

Sl No	Symptoms	n (%)
1.	Fever	42 (42)
2.	Cough	86 (86)
3.	Haemoptysis	17(17)
4.	Chest pain	10 (10)
5.	Weight loss	12(12)
6.	Weakness	57(57)

Table 2: Distribution of patients according to risk factors (N=4)

Sl No	Risk factor	n (%)
1.	HIV	3 (3)
2.	Organ transplant	1 (1)
3.	Ingestion of raw or undercooked crab or crayfish	0

Table 3: Prevalence of nocardiosis and paragonimiasis in clinically suspected tuberculosis patients (N=100)

Sl No	Organism	No. of patients	ZN stain positive	Kinyoun stain positive	Culture positive	Wet mount	%
1.	Nocardia spp.	100	1	1	1	-	1
2.	Paragonimus spp.	100	-	-	-	0	0

Table 4: Prevalence of nocardiosis in suspected tuberculosis patients who are immunocompromised (N=4)

Organism	ZN stain positive n (%)	Kinyoun stain positive n (%)	Gram stain positive n (%)	Growth in McClung's broth n (%)	Culture positive n (%)
Nocardia spp.	1 (25)	1 (25)	1 (25)	1 (25)	1 (25)

Out of the 100 clinically suspected tuberculosis patients, four were identified as immunocompromised due to factors such as HIV infection or organ transplantation. Among these immunocompromised patients, Nocardia spp. was detected in one patient, highlighting a potential increased susceptibility to nocardial infections in individuals with weakened immune systems. This finding underscores the importance of considering opportunistic infections, such as Nocardia spp., in immunocompromised patients presenting with symptoms resembling tuberculosis [Table 4].

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DISCUSSION

This study aimed to investigate the prevalence of pulmonary paragonimiasis and nocardiosis among clinically suspected pulmonary tuberculosis (PTB) patients who were negative for Mycobacterium tuberculosis on smear and molecular testing. Notably, Nocardia spp. was identified in 1% of cases, while no evidence of Paragonimus spp. infection was found. These results underscore the low prevalence of these infections in the study population but highlight the diagnostic importance of considering alternative infections in patients presenting with tuberculosis-like symptoms.[11]

In fact, patients with paragonimiasis or nocardiosis were often mistaken for having tuberculosis due to similar symptoms like fever, cough, hemoptysis, and chest pain. It has been recorded that many patients who had received therapy for tuberculosis, were later diagnosed with paragonimiasis as evident from a case report from Manipur by Singh TS et al,[12] where 23 (58.9%) out of 39 patients treated for tuberculosis, were eventually diagnosed with paragonimiasis. Similarly, Narain K et al,[13] reported 23 paragonimiasis cases of which 16 were misdiagnosed with PTB on the basis of chest X-ray and clinical history and were treated with the RNTCP Category III regimen, with no response. These observations emphasize the need to consider paragonimiasis in the differential diagnosis of TB in areas where the potential for tuberculosis and paragonimiasis co-exist.

The demographic distribution showed a predominance of males (61%) and older adults (40–49 years and ≥60 years age groups). The geographic representation was primarily from the Imphal West district, likely reflecting the study's healthcare accessibility or referral patterns.

A 1993 study by Singh TS et al,[12] reported Paragonimus spp. eggs were detected in 4 sputum samples out of 75 sputum samples of individuals positive for paragonimiasis skin test using the wet mount method thus giving the recovery rate of eggs to be 5.33%, which is low. Initially, a total of 3,467 individuals were screened for paragonimiasis using an intradermal test with a saline extract of adult P. westermani as a test antigen and found 234 individuals positive for the skin test giving a prevalence of paragonimiasis to be 6.7% in Manipur. In a similar study by Devi KR et al,[14] conducted in 2007 at Changlang district of Arunachal Pradesh, a prevalence rate of 11.1% (75/675 patients) for paragonimiasis was observed when using the wet mount method whereas the prevalence rate was higher when ELISA was used as the screening method on the same participants i.e., 31.5% (213/675 patients). On the contrary, in the present study, out of 100 samples processed, using only the wet mount method, no eggs of Paragonimus spp. were detected.

In the present study, nocardiosis was diagnosed in 1% (1/100) of patients, similar to a 2000 study by Singh TM et al,[15] in Amritsar, India, which reported a 1.6% prevalence (16/1016) among suspected tuberculosis patients. Also, Ekrami A et al,[16] in 2014 in Iran found a similar prevalence (1.07%) amongst a total of 157 suspected tuberculosis patients in their study. But higher prevalence of nocardiosis have been reported in some studies conducted in other parts of the world like Tanzania and Iran by Hoza AS et al,[17] in 2017 and Bafghi MF et al,[18] in 2015, respectively. Hoza AS et al,[17] found a prevalence of 5.4% for nocardiosis among 372 patients suspected with tuberculosis and Bafghi MF et al,[18] reported a prevalence of 5.3% among 517 participants who were suspected tuberculosis patients which is much higher than the present study.

Cough and fever were the most common clinical complaints, consistent with respiratory infections. However, the absence of undercooked crab consumption in the cohort suggests that traditional dietary habits may not significantly contribute to paragonimiasis in this region, contrary to its established epidemiology in other endemic areas.

Immunocompromised status, present in 4% of the participants, was a critical factor influencing infection susceptibility. One HIV-positive patient was diagnosed with nocardiosis, indicating an increased vulnerability to opportunistic infections in such individuals. This finding reinforces the necessity of tailored diagnostic approaches for immunocompromised patients with unexplained pulmonary symptoms. In this study, the patient diagnosed with nocardiosis was a post-kidney transplant recipient on immunosuppressive therapy without proper follow-up, who ultimately succumbed to septic shock. Nocardia spp. is known to commonly affect immunocompromised individuals, particularly organ transplant recipients, as reported by Devi KR et al,[14] in 2007—the first case of nocardiosis in Manipur involving a kidney transplant patient.

The prevalence of nocardiosis among immunocompromised patients varies between 2–20% across studies. In the present study out of four immunocompromised patients, one patient is positive for nocardiosis in the present study giving the prevalence rate 25% among immunocompromised patients, in contrary, a study by John GT et al,[19] in south India in 2002 over a period of 30 years out of 1968 post kidney transplant patients 27 found to be positive for nocardiosis giving the prevalence of 1.37% in post kidney transplant patients. Though Hoza AS et al,[17] in Tanzania in 2017 reported a 25% prevalence among immunocompromised patients in their study which is similar to our study. On the contrary Ekrami A et al,[16] and Alnaum HMA et al,[20] in their study, found 6.25% and 11.76% prevalence respectively in immunocompromised patients. The comprehensive diagnosis used direct wet mount microscopy, staining techniques, and culture methods, displaying a high specificity for Nocardia spp. Kinyoun staining provided a key observation such as identification of branching, acid-fast bacilli.[21] These techniques validated the

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presence of Nocardia spp. highlighted the necessity of incorporating staining and culture techniques into routine diagnostics, particularly in developing nations, where sensitive molecular tools may not always be accessible. However, the findings may not be generalized due to few limitations like small sample size and narrow focus on clinically suspected TB cases. In addition, the use of self-reported dietary histories may have led to recall bias.

CONCLUSION

This study underlines the importance to keep in mind nocardiosis in the differential diagnosis of chronic TB-like pulmonary infections, particularly in immunocompromised patients. Though the lack of the clinical picture of paragonimiasis implies low regional prevalence, these findings necessitate continued clinical surveillance efforts and diagnostic vigilance towards such emerging spectrum of pulmonary infections from the northeastern part of India. Implementing more advanced diagnostic methods and personalized public health interventions will be key to controlling these infections.

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