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CORRELATION OF SPUTUM AFB, MANTOUX TEST AND PCR IN NON-TUBERCULOSIS MYCOBACTERIUM

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ABSTRACT:
The purpose of the study was done to observe correlation of AFB, Monteux test and PCR in non-tuberculosis mycobacterium for the detection and identification of clinically most frequent NTM at the species. A Prospective Experimental Study was done on 50 IP and OP patients for duration of 12 months who presented to various Departments of VMMC, Karaikal with signs and symptoms of Tuberculosis. Patients of All Age Group with clinical suspicion of Tuberculosis, Both New and Retreatment Tuberculosis Patients were included in the study. Microbiologically confirmed case of Mycobacterium Tuberculosis were excluded from the study. Out of 50 (100%) cases who took part in the study, sample type was distributed among 49 (98.0%) sputum samples and 1 (2.0%) bronchial wash. Out of 50 (100%) cases who took part in the study to determine the prevalence of Acid-Fast Bacilli (AFB), 24 (48.0%) of cases had Acid-Fast Bacilli and 26 (52.0%) had no Acid-Fast Bacilli in staining. Out of 50 (100%) cases who took part in the study to determine the prevalence of tuberculosis by MANTOUX screening, 37 (74.0%) tested positive for MANTOUX and 13 (26.0%) tested negative for MANTOUX. Out of 50 (100%) cases who took part in the study to determine the detection of genes by Polymerase Chain Reaction (PCR) test, 36 (72.0%) of cases had specific genes and 14 (28.0%) of cases had no specific genes detected by polymerase chain reaction. Out of 50 (100%) cases who took part in the study to determine the prevalence of Acid-Fast Bacilli and its gene by Polymerase Chain Reaction, 6 (42.9%) and 20 (55.6%) cases tested negative for acid-fast bacilli staining, 8 (57.1%) and 16 (44.4%) cases tested positive for acid-fast bacilli staining. For gene detection by polymerase chain reaction 6 (42.9%) and 8 (57.1%) cases had no specific genes where 20 (55.6%) and 16 (44.4%) of cases had specific genes. Out of 50 (100%) cases who took part in the study to the determine the prevalence of MANTOUX and its gene by Polymerase Chain Reaction prevalence, 2 (14.3%) and 11 (30.6%) of cases tested negative for MANTOUX test whereas 12 (85.7%) and 25 (69.4%) of cases tested positive for MANTOUX test. For specific gene detection by polymerase chain reaction 2 (14.3%) and 12 (85.7%) of cases had no specific genes but 11 (30.6%) and 25 (69.4%) of cases had specific genes suggesting that PCR is a technique of convenience.

INTRODUCTION:

The incidence and prevalence of diseases that are caused by NTM are continuing to rise all over the world, which has led to the NTM's emergence as major human pathogens in modern times. Mycobacterium avium complex (MAC) is composed of Mycobacterium avium, Mycobacterium intracellulare, and Mycobacterium chimaera. Mycobacterium abscessus complex is composed of Mycobacterium abscessus subspecies bolletii, subspecies massiliense, and Mycobacterium chelonae. Mycobacterium kansasii is the third most common species that is frequently isolated from patients The diagnosis and distinction of NTM from MTBC is of significant diagnostic importance due to the fact that the pathophysiology and treatment protocols for both illnesses are distinct from one another. Even among NTM of the same species complex, various treatment techniques are required because to the variable sensitivity pattern towards anti-TB medicines. Therefore, the quick distinction of MTBC from NTM and the species-specific detection of NTM is essential for effective patient care and therapy in order to achieve optimal results. In most cases, the frequency of NTM infections has been notified from TB non-endemic countries, and only very rarely from TB endemic countries. This is due to the fact that the likelihood of missing NTM infections is greater in TB endemic countries. Because the current standard of care for diagnostic testing does not include bacterial characterisation, smear-

positive NTM patients are often incorrectly labelled as MTBC. Therefore, the majority of cases of NTM infection either go undiagnosed or are treated with chemotherapy, which is typically reserved for TB patients. This leads to the development of NTM strains that are resistant to medication treatments. In light of this, the purpose of the study was done to observe correlation of AFB, Monteux test and PCR in non-tuberculosis mycobacterium for the detection and identification of clinically most frequent NTM at the species.

AIM AND OBJECTIVES:

Genotypic Identification of Atypical Mycobacterium Species from clinical samples collected from Presumptive Tuberculosis Patients and to observe correlation of AFB, Monteux test and PCR in non-tuberculosis mycobacterium for the detection and identification of clinically most frequent NTM at the species.

MATERIALS AND METHODS:

A Prospective Experimental Study was done on 50 IP and OP patients for duration of 12 months who presented to various Departments of VMMC, Karaikal with signs and symptoms of Tuberculosis. Patients of All Age Group with clinical suspicion of Tuberculosis, Both New and Retreatment Tuberculosis Patients were included in the study. Microbiologically confirmed case of Mycobacterium Tuberculosis were excluded from the study.

RESULTS:

Table 1: Gender Distribution

Variables	Frequency	Percent
Female	11	22.0%
Male	39	78.0%
Total	50	100.0%

Fig 1: Distribution of Sample Type

Out of 50 (100%) cases who took part in the study, sample type was distributed among 49 (98.0%) sputum samples and 1 (2.0%) bronchial wash

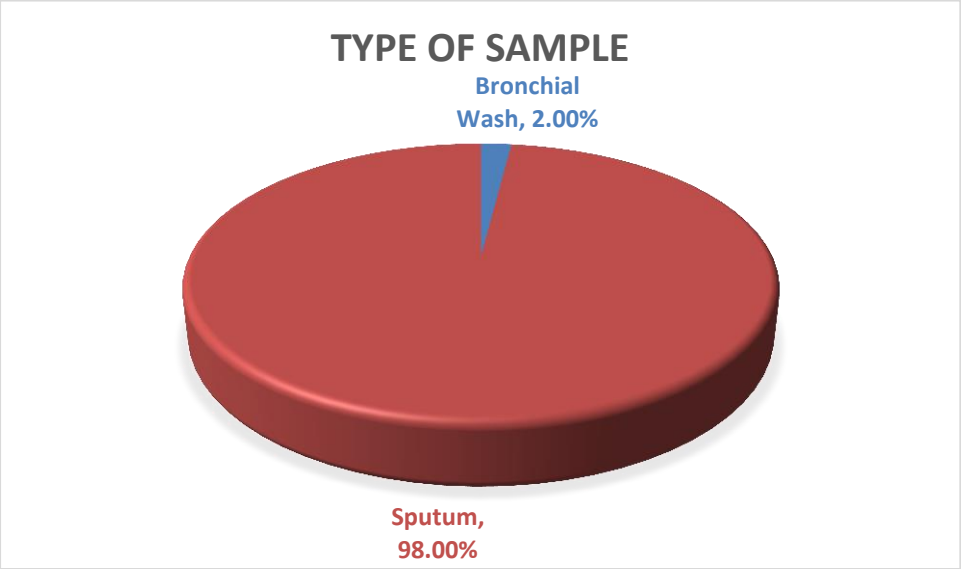
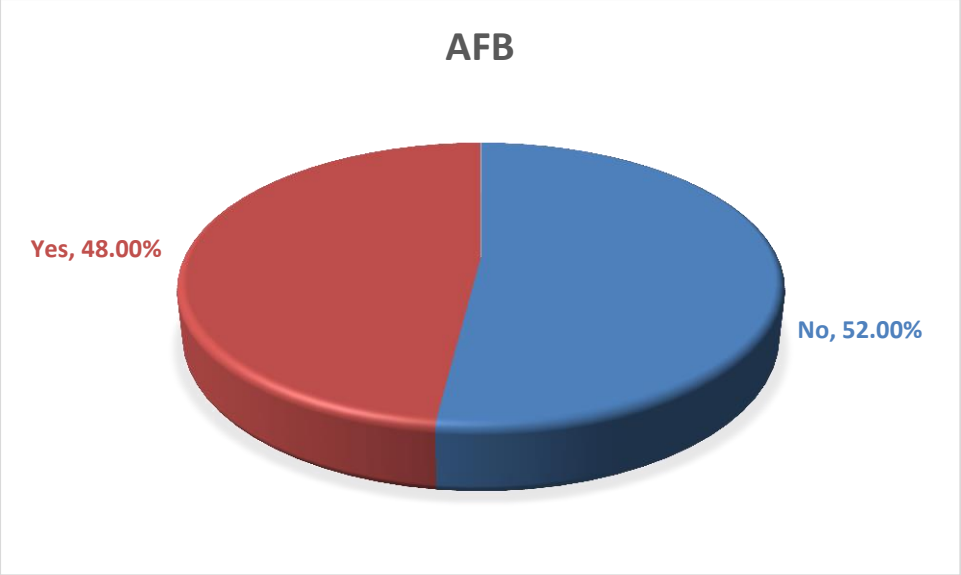
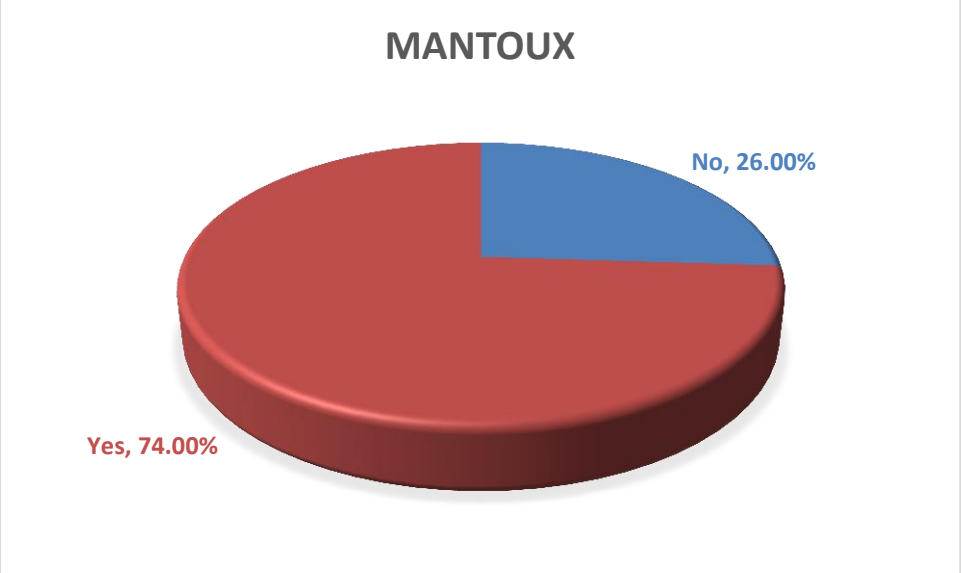


Fig 2: Distribution of Acid-Fast Bacilli



Out of 50 (100%) cases who took part in the study to determine the prevalence of **Acid-Fast Bacilli (AFB), 24 (48.0%) of cases had Acid-Fast Bacilli and 26 (52.0%) had no Acid-Fast Bacilli in staining.**

Fig 3: Distribution of MANTOUX

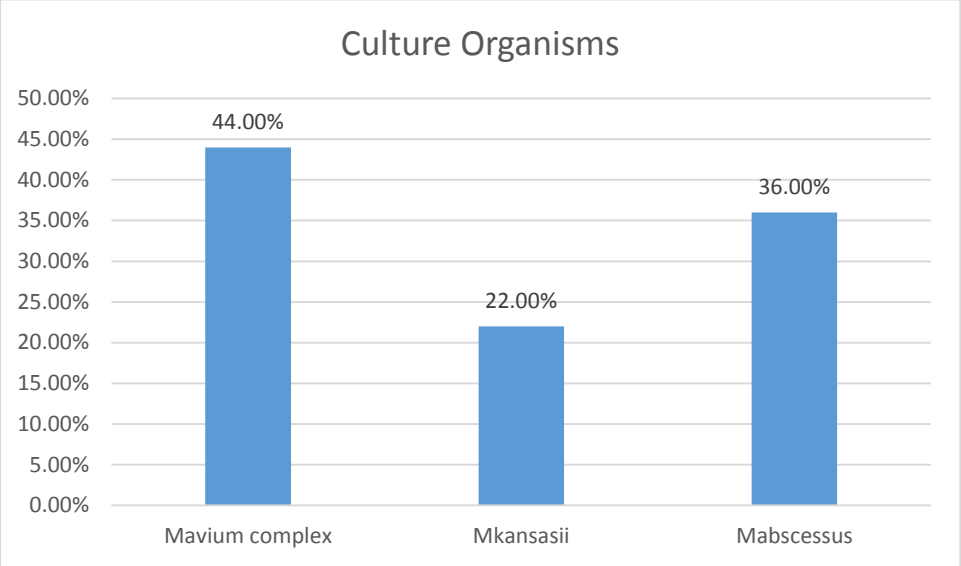


Out of 50 (100%) cases who took part in the study to determine the prevalence of tuberculosis by MANTOUX screening, 37 (74.0%) tested positive for MANTOUX and 13 (26.0%) tested negative for MANTOUX.

Table 2: Distribution of Culture Organisms

Culture Organisms	Frequency	Percent
***M. avium* complex**	22	44.0%
M. kansasii	11	22.0%
M. abscessus	18	36.0%

Fig 4: Distribution of Culture Organisms



Out of 50 (100%) cases who took part in the study to determine the prevalence of culture organisms. 22 (44.0%) of *M. avium* complex growth, 18 (36.0%) of *M. abscessus* growth and 11 (22.0%) of *M. kansasii* was observed.

Table 3: Distribution of PCR

PCR	Frequency	Percent
No	14	28.0%
Yes	36	72.0%
Total	50	100.0%

Out of 50 (100%) cases who took part in the study to determine the detection of genes by Polymerase Chain Reaction (PCR) test, 36 (72.0%) of cases had specific genes and 14 (28.0%) of cases had no specific genes detected by polymerase chain reaction**.**

Table 4: Correlation of Acid-Fast Bacilli by PCR

AFB	PCR		P Value
	No	**Yes**	
No	**6**	**20**	**0.420**
	42.9%	**55.6%**	
Yes	**8**	**16**	
	57.1%	**44.4%**	
Total	**14**	**36**	
	100.0%	**100.0%**	

Out of 50 (100%) cases who took part in the study to determine the prevalence of Acid-Fast Bacilli and its gene by Polymerase Chain Reaction, 6 (42.9%) and 20 (55.6%) cases tested negative for acid-fast bacilli staining, 8 (57.1%) and 16 (44.4%) cases tested positive for acid-fast bacilli staining. For gene detection by polymerase chain reaction 6 (42.9%) and 8 (57.1%) cases had no specific genes where 20 (55.6%) and 16 (44.4%) of cases had specific genes.

Table 5: Correlation of MANTOUX by PCR

MANTOUX	PCR		P Value
	No	**Yes**	
No	**2**	**11**	**0.239**
	14.3%	**30.6%**	
Yes	**12**	**25**	
	85.7%	**69.4%**	

Total	**14**	**36**	
	100.0%	**100.0%**	

Out of 50 (100%) cases who took part in the study to the determine the prevalence of MANTOUX and its gene by Polymerase Chain Reaction prevalence, 2 (14.3%) and 11 (30.6%) of cases tested negative for MANTOUX test whereas 12 (85.7%) and 25 (69.4%) of cases tested positive for MANTOUX test. For specific gene detection by polymerase chain reaction 2 (14.3%) and 12 (85.7%) of cases had no specific genes but 11 (30.6%) and 25 (69.4%) of cases had specific genes.

DISCUSSION:

The conventional methods for laboratory diagnosis of Atypical Mycobacteria include microscopy (acid fast staining) and culture. For enhancing treatment strategies and reducing the potential of spreading Atypical Mycobacterial diseases in a community new diagnostic method for Atypical Mycobacteria complex are needed to help combat this deadly disease, in which the use of nucleic acid amplification and detection in sputum, blood and body fluids may provide quick and specific results for identifying the Atypical Mycobacteria complex. Amongst 50 (100%) cases who took part in the study, 39 (78.0%) of them were male and 11 (22.0%) of them were female cases. When compared with female cases male cases were highest in number. Selassie Ameke et al., study, thirty-five (30.4%) of the cases were females and the remaining 80 (70.1%) being males. Out of 50 (100%) cases who took part in the study, sample type was distributed among 49 (98.0%) sputum samples and 1 (2.0%) bronchial wash. Can Biçmen et al., done the identification of atypical mycobacteria in sputum samples. Ahmed Gaballah et al identified atypical mycobacteria from the clinical specimens consisted of sputum (22), lymph node aspirates (2), urine (3), and serum (3). Out of 50 (100%) cases who took part in the study to determine the prevalence of Acid-Fast Bacilli (AFB), 24 (48.0%) of cases had Acid-Fast Bacilli and 26 (52.0%) had no Acid-Fast Bacilli in staining. Fabiane N. Riello et al., found that 38 % of the MTB positive samples were AFB negative, whereas 72 % of the NTM infections were AFB negative. In Ahmed Gaballah Out of 27 specimens, 23 were AFB positive for the diagnosis of NTM. Out of 50 (100%) cases who took part in the study to determine the prevalence of tuberculosis by MANTOUX screening, 37 (74.0%) tested positive for MANTOUX and 13 (26.0%) tested negative for MANTOUX. A. J. KEAY M.B. Edin et al., done the demonstration of 7 cases of atypical mycobacterial infection. Differential Mantoux testing would appear to be the most satisfactory clinical method of separating atypical mycobacterial infection from tuberculosis according to their conclusion. Out of 50 (100%) cases who took part in the study to determine the prevalence of culture organisms. 22 (44.0%) of M. avium complex growth, 18 (36.0%) of M. abscessus growth and 11 (22.0%) of M. kansasii was observed.Out of 50 (100%) cases who took part in the study to determine the prevalence of Acid-Fast Bacilli and its gene by Polymerase Chain Reaction, 6 (42.9%) and 20 (55.6%) cases tested negative for acid-fast bacilli staining, 8 (57.1%) and 16 (44.4%) cases tested positive for acid-fast bacilli staining. For gene detection by polymerase chain reaction 6 (42.9%) and 8 (57.1%) cases had no specific genes where 20 (55.6%) and 16 (44.4%) of cases had specific genes. Out of 50 (100%) cases who took part in the study to the determine the prevalence of MANTOUX and its gene by Polymerase Chain Reaction prevalence, 2 (14.3%) and 11 (30.6%) of cases tested negative for MANTOUX test whereas 12 (85.7%) and 25 (69.4%) of cases tested positive for MANTOUX test. For specific gene detection by polymerase chain reaction 2 (14.3%) and 12 (85.7%) of cases had no specific genes but 11 (30.6%) and 25 (69.4%) of cases had specific genes.P-value is 0.420 which is statistically insignificant**.**

CONCLUSION:

In conclusion, multiplex PCR is a simple, fast, convenient and reliable technique for identification of NTM species in the routine laboratory. This method can be used in developing countries for identification of most common NTM from pulmonary samples. To the best of our knowledge this is the first type of study conducted in India. In conclusion, although the NTM culture and identification methods used are still traditional, there is increasing focus on NTM.

References

1. **Field SK, Cowie RL. Lung disease due to the more common nontuberculous mycobacteria. Chest. 2006 Jun 1;129(6):1653-72.**
2. **Agrawal V, Patgaonkar PR, Nagariya SP. Tuberculosis of spine. Journal of craniovertebral junction and spine. 2010 Jul;1(2):74.**
3. **Winburn B, Sharman T. Atypical mycobacterial disease. InStatPearls [Internet] 2022 Jan 14. StatPearls Publishing.**
4. **Winburn B, Sharman T. Atypical mycobacterial disease. InStatPearls [Internet] 2022 Jan 14. StatPearls Publishing.**
5. **Pavlik I, Ulmann V, Hubelova D, Weston RT. Nontuberculous mycobacteria as sapronoses: A review. Microorganisms. 2022 Jul 3;10(7):1345.**
6. **Gubernot D, Jazwa A, Niu M, Baumblatt J, Gee J, Moro P, Duffy J, Harrington T, McNeil MM, Broder K, Su J. US Population-Based background incidence rates of medical conditions for use in safety assessment of COVID-19 vaccines. Vaccine. 2021 Jun 23;39(28):3666-77.**
7. **Balkhair AA, Al-Muharrmi ZK, Ganguly S, Al-Jabri AA. Spectrum of AIDS defining opportunistic infections in a series of 77 hospitalised HIV-infected Omani patients. Sultan Qaboos University Medical Journal. 2012 Nov;12(4):442**
8. **Jarzembowski JA, Young MB. Nontuberculous mycobacterial infections. Archives of pathology & laboratory medicine. 2008 Aug;132(8):1333-41.**
9. **Koh WJ. Nontuberculous mycobacteria—overview. Microbiology spectrum. 2017 Jan 27;5(1):5-1.**
10. **Stout JE, Koh WJ, Yew WW. Update on pulmonary disease due to non-tuberculous mycobacteria. International Journal of Infectious Diseases. 2016 Apr 1;45:123-34.**
11. **Furuuchi K, Morimoto K, Yoshiyama T, Tanaka Y, Fujiwara K, Okumura M, Izumi K, Shiraishi Y, Mitarai S, Ogata H, Kurashima A. Interrelational changes in the epidemiology and clinical features of nontuberculous mycobacterial pulmonary disease and tuberculosis in a referral hospital in Japan. Respiratory Medicine. 2019 Jun 1;152:74-80.**
12. **Van Ingen J, Bendien SA, De Lange WC, Hoefsloot W, Dekhuijzen PR, Boeree MJ, Van Soolingen D. Clinical relevance of non-tuberculous mycobacteria isolated in the Nijmegen-Arnhem region, The Netherlands. Thorax. 2009 Jun 1;64(6):502-6.**