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ROLE OF MYCOBACTERIAL CULTURE IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS

Medicine

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ABSTRACT

Inspite of the introduction and developments of new molecular diagnostic techniques, the gold standard for mycobacterial diagnosis is still culture. Most of the studies on mycobacteria have been focused on pulmonary infection, while extrapulmonary infection has poorly been explored.

AIM: To isolate Mycobacteria from extrapulmonary tuberculosis cases by automated and conventional method.

SETTING AND DESIGN: This study on extra-pulmonary tuberculosis was conducted in the department of Microbiology, Medical College, Kozhikode for a period of one year.

MATERIALS AND METHODS: A total of 118 specimens were included in the study. History, clinical examination findings and results of relevant investigations were obtained and recorded in a proforma. All specimens were processed by modified Petroff's method and subjected to smear microscopy. The processed specimens were cultured by both automated and conventional methods.

RESULTS: Isolation rate by automated method was 14(11.86%) whereas by conventional method it was 4(3.38%). Of total culture positives only 4(3.38%) were smear positive. Isolation from smear negative specimens was 10(8.77%) by automated method and 1(0.87%) by conventional method. HIV- TB co-infection was seen in 15 (12.71%) cases.

CONCLUSION: Mycobacterial culture and its identification provides a definitive diagnosis of extrapulomary tuberculosis especially in smear negative TB. It also provides isolates for drug sensitivity testing

KEYWORDS

Extrapulmonary , Mycobacteria, Tuberculosis, LJ medium, Automated

INTRODUCTION

Tuberculosis remains a major public health problem worldwide. World health organization estimates that 10.4 million of tuberculosis cases and 1.6 million deaths are directly attributable to the disease annually[1]. Of the 6.3 million new TB cases recognized by WHO in 2017, 16% were extrapulmonary TB cases. In endemic areas, especially in countries with a high HIV prevalence, extra pulmonary TB contributes significantly to disease burden. It causes significant morbidity and mortality, where its significance has increased progressively over the past few years[2].

India is one of the most affected countries. Moreover there is increasing incidence of drug resistant tuberculosis.The symptoms of EPTB is vague and thus high index of suspicion is required to diagnose. Early diagnosis plays an important role in control of tuberculosis. The pauci-bacillary nature of disease and non-uniform distribution of the bacteria may lead to false negative results for most of the laboratory procedures[3]. Culture techniques have been estimated to detect as low as 10-100 bacilli/ml[4]. Sensitivity 80-85%; Specificity 98%. It is required for precise identification of causative organisms. Moreover it also help in doing drug susceptibility testing of the isolates obtained, which cannot be done by many of the rapid detection techniques available today. Mycobacterial culture also aids in species identification.

MATERIALS AND METHODS

This study on extra-pulmonary tuberculosis was conducted in the department of Microbiology, Medical College, Kozhikode for a period of one year. The study was approved by our Institutional Ethics Committee A total of 118 specimens were included in the study. History, clinical examination findings and results of relevant investigations were obtained and recorded in a proforma. All specimens were collected under universal aseptic precaution in suitable sterile containers. Lymph node aspirate, biopsy specimen, bone marrow, CSF, urine, ascitic fluid and pleural fluid were collected in sterile leak proof containers provided by the Microbiology department and processed; In case of any delay, sample was refrigerated and processed within 24 hours.

All specimens were processed in Biosafety cabinet class II. Direct smears prepared from of all specimens and Ziehl-Neelsen staining was done. Processing was done by modified Petroff's method[5]. In the case of sterile fluids, the specimen was transferred into a 50 ml sterile conical bottom graduated centrifuge tubes. Equal volume of 4% NAOH was added, Vortexed for 15-30 seconds and incubated at room temperature for 10 minutes. The samples were agitated during incubation for better liquefaction. Distilled water was added up to 45ml mark of centrifuge tube. The tube was closed tightly. It was mixed by just inverting the tube and centrifuged at 3000g for 20 minutes. The supernatant was decanted and sediment re suspended with 1ml of distilled water. For biopsy and bone marrow specimen they were added aseptically into sterile mortar. The specimen was ground with minimal volume of distilled water by using sterile pestle, and then decontaminated using Modified Petroff's method. Urine samples were first centrifuged for 15 minutes at 3000g .The supernatant was decanted and proceeded like body fluids. CSF being from sterile site, was not decontaminated.

The processed samples were simultaneously inoculated into MB/Bact bottle and two slopes of LJ medium. Each MB/Bact bottle was labeled with appropriate patient information. The bottles were equilibrated to room temperature before inoculation. Septum was disinfected with alcohol and allowed to dry.0.5ml of reconstituted MB/BacT antibiotic supplement (MAS) was added to culture bottle for non sterile specimens and for sterile specimens 0.5 ml of MB/BacT Reconstitution fluid was added as such. Then 0.5ml of the processed sample was added. Inoculation was done through the rubber septum by means of a syringe. The inoculated bottles were loaded into MB/BacT instrument. The processed samples which were inoculated into two slopes of LJ medium are incubated at 37°C. BacT/ALERT MP bottles which flagged positive by the instrument were unloaded. The large clumps were broken by vortexing and were suspended uniformly. A small portion of broth was draw from positive bottle by using sterile syringe and needle. Two smears were made, one for acid fast staining and one for gram stain. Growth of Acid fast bacilli was confirmed by Ziehl-Neelsen staining. Subculture was done on LJ medium and blood agar from positive bottles. BacT/ALERT MP Bottles were declared negative for AFB after 42 days of incubation and the corresponding LJ slopes after 12 weeks of incubation[6].

RESULTS

During the study period of 1 year total of 118 samples from the various extra pulmonary sites were proceeded for mycobacterial culture by automated and conventional methods. These included specimens includes CSF, bonemarrow, lymph node aspirate, ascitic fluid, pus , pleural fluid, colonic biosy, psoas abscess, skin biopsy, hydrocele fluid, synovial joint biopsy, liver biopsy. [Table 1].

Table 1: Isolation of Acid fast bacilli from different specimens by Automated and Conventional method

Specimen	Number	Isolation by MB/Bact	Isolation by LJ medium

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CSF	27	4	-
Bone marrow	17	-	-
Urine	13	-	-
Lymph node aspirate	9	2	1
Ascitic fluid	9	2	1
Pus	8	-	-
Pleural fluid	7	1	-
Colonic biopsy	7	2	1
Psoas abscess	5	1	-
Skin biopsy	5	-	-
Hydrocele fluid	1	1	1
Cerebellar abscess	1	1	-
Synovial joint biopsy	1	-	-
Liver biopsy	1	-	-
Total	118	14	4

80 (67.79%) were from males and 38 (32.20%) were from females. Males dominated in the study population with male: female ratio 2.1:1. Maximum number of patients belonged to 20-40 age group with mean age 37.9 ± 1.3 years. Isolation rate by automated method was 11.86% whereas by conventional method it was only 3.38% [Figure 1].

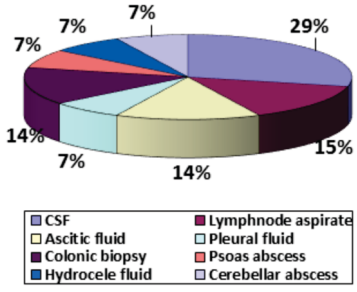

Fig.1: Isolates obtained by MB/Bact culture (n=14)

In smear positive samples the percentage of isolation was 80% by MB/BacT and 20 % by LJ medium Out of 5 smear positive cases, 3 (75%) yielded pure growth of Mycobacteria in both LJ medium and MB/BacT. This explains that load or quantum of the organism is an important determinant. One sample which grew in MB/BacT failed to grow in LJ medium. One sample of smear positive cases showing no growth in both medium may be explained by effects of antitubercular agents. While among 114 smear negative samples, MB/BacT alone detected Mycobacteria in 10 samples and L-J media in 1sample only. In smear negative samples isolation was 8.77% by MB/BacT and 0.87% by LJ medium. [Table 2].

Table 2: Isolation rate of mycobacteria by MB/Bact system and LJ medium in smear positive and negative cases

Specimen (n = 14)	Isolation by MB/Bact culture	Isolation by LJ medium culture
Smear positive	4	3
Smear negative	10	1

MTB was differentiated from Non-tuberculous mycobacterium by heat stable catalase test. Of the total isolates, one each of ascitic fluid and colonic biopsy were heat stable catalase positive. HIV- TB co-infection was seen in 15 (12.71%) cases. Of the overall 14 positive extrapulmonary cases 8 (57.14%) were from HIV positive patients.

DISCUSSION

The burden of TB in India is indeed staggering by any measure. In the present scenario TB was declared a "global emergency" by WHO due of its toll on the health of individuals and its social and economic impact on overall development of a country.

The continuing global threat of tuberculosis has led to an urgent need to design more effective diagnostic procedures and develop improved antituberculous therapies. Despite the introduction of molecular diagnostic techniques, the gold standard for mycobacterial diagnosis is still culture. Most of the studies on mycobacteria have been focused on pulmonary infection, while extrapulmonay infection has poorly been explored.

A total of 118 samples from the various extra pulmonary sites were proceeded for mycobacterial culture by automated and conventional methods. Males dominated in the study population with male: female ratio 2.1:1 females 33%, and male: female ratio 2:1 . According to global tuberculosis report 2018 the male:female ratio among adults is approximately 2:1. The difference was due to differences in the societal roles of men and women that influence their risk of exposure to TB and access to medical care (gender differences). The social stigma of the disease adds to the burden for both men and women. Studies indicate that while men have to deal with the stigma at their workplaces and in the community, women are ostracised in the household and neighbourhood.

In this study maximum numbers of patients were in age group 20- 40 years (42.37%) and the mean age was 37.9 ± 1.3 years. In another study conducted by Pang Y, An J and Shu W in 2008- 2017 maximum number of patients in 25-44 age group[7] . Our study correlates well with a study conducted by Soumithesh Chakravorthy et al in 2003[8] where the maximum number of samples were from patients in the age group of 20-39 years and the mean age was 35 years. In the present study maximum number of cases occurred in the economically productive age group. In Kerala, where tuberculosis is endemic the most active mobile elements of the society are at increased risk of contacting the disease. The risk of being infected as well as the risk of infection progressing to active disease is more in that age group.

In the present study the isolation rate was 11.86 % by automated method and 3.38 % by LJ culture method . .Highest isolation rate was from hydrocele fluid and cerebellar abscess, where the single specimen processed was positive. The isolation rate from colonic biopsy was 28.57%, that of lymph node aspirate and ascitic fluid were 22.22%. The isolation rate was 20% from psoas abscess, 14.81% from CSF, and 14.2% from pleural fluid. No isolation could be obtained from bone marrow, urine, skin biopsy, synovial joint biopsy and liver biopsy. In a study conducted by Anastacio Palacio in 2018 the rate of isolation was 5.5 % which is lower than the present study[9]. S Rishi et al [10]found the recovery rate was 21.98% by automated and 4.96% by conventional. The paucibacillary nature of extrapulmonary tuberculosis is the reason for low yield in culture. More than that the extrapulmonary specimens needs decontamination methods which can sometimes be harmful for mycobacteria. The low diagnostic yield by culture confirmed the findings of earlier studies on extrapulmonary TB diagnosis.

In our study the isolation rate by automated method was 28.49% more than that by LJ method. In a study by S Rishi et al in 2004[10] the isolation rate by automated culture was 34.11% more than conventional method. It is seen that comparative studies of this type bear two major biases which cannot, however, be eliminated in a routine clinical laboratory, where standardized protocols for cultures must be strictly followed. First, the study design could account for the decreased sensitivity of the LJ slants since each slant received approximately 0.1 ml of specimen versus the 0.5-ml inoculated into MB/BacT and second, the reading frequency was not the same for both media. For automated, reading was done daily and for LJ media once weekly.

In the present study, isolation rate among smear positive specimen was 80% by MB/BacT and 20% by L J medium. Out of 5 smear positive cases, 3 (75%) yielded pure growth of Mycobacteria in both LJ medium and MB/BacT. This explains that load or quantum of the organism is an important determinant. One sample which grew in MB/BacT failed to grow in LJ medium. One sample of smear positive cases showing no growth in both medium may be explained by effects of antitubercular agents. For these treated cases addition of antimicrobial agent inactivating substances such as charcoal, Fuller's earth, or resins to culture media for Mycobacteria may provide more reliable results. In a study conducted by Dr Shabana 6% was the culture positivity in smear negative extrapulmonary TB [11]. Whereas in a study by Conception F.Ang et al in Phillipines[12] rate of isolation was 89.4% for MB/BacT and 85.2% for LJ. Mahadev et al [13]reported isolation of 81% and 80% respectively.

While among 114 smear negative samples, MB/BacT alone detected Mycobacteria in 10 samples and L-J media in 1sample only. In a study conducted by Conception F. Ang et al[12] in Philippines in 2001 among smear negative specimen, mycobacterial growth was noted to be 10.9% by MB/BacT system and 7.3% by LJ method. In another study by Mahadev et al[13] the percentage isolation was 15.79% and 13% respectively MB/BacT was proven to be more efficient than LJ

medium in culture isolation of mycobacteria for smear negative and smear positive specimens as seen in our study. This is more evident with smear negative specimens. So automated culture is more sensitive in smear negatives.

Out of the total 118 patients, 15 (12.71%) of people had positive HIV status. Out of the total 15 HIV positive people 8 (53.33%) had positive culture. Estimates of TB-HIV co-infection prevalence ranged from 6 to 52.1%[14] .In a study by Ayyagiri et al[15] the proportion of extrapulmonary tuberculosis was 25% among HIV infected groups. In another study by BK Dash et al[16] proportion of extrapulmonary tuberculosis among HIV infected persons was 75%. So all cases of extra-pulmonary tuberculosis should be screened for co-existing HIV infection and vice versa, for early diagnosis of infection and appropriate treatment. In our study the specimens of all HIV positives were smear negative. All the isolation was obtained by automated culture. So it is seen that automated culture is more sensitive in HIV positives. Diagnosis of TB in HIV positive patients is more difficult than HIV negatives because of the atypical presentation[17].

Of the 118 cases extra pulmonary tuberculosis with pulmonary lesion was seen in 18 (15.25 %) patients .Acid fast bacilli was isolated from extrapulmonary specimens in 5 (27.77%) of these 18 cases. In a study by Tanyalak Parimon[18] et al the diagnosis of pulmonary tuberculosis was confirmed by culture in 21.05% patients with extrapulmonary tuberculosis. Although it is not routine practice to perform AFB sputum examination in otherwise immunocompetent extrapulmonary tuberculosis patients with normal chest imaging results, sputum examination may nonetheless identify subclinical involvement of the respiratory system with TB.

Of the total 118 patients twenty patients (16.94%) received ATT. Of the 20 patients, isolation was obtained in 7(35%) cases. In the 7 culture positives, two patients were started on ATT with clinical suspicion before sending samples for culture. 5 patients were already on ATT for pulmonary tuberculosis. Of the 14 culture positives, those 7 patients who were not on treatment were put on ATT after sending the samples for culture. Of the total 20 patients on ATT, five patients (40%) received anti tuberculous treatment for a duration of six to nine months and 11 patients (61.11%) were treated for nine to 12 months. One patient was treated for more than one year. Three patients had not completed the full course of treatment. Two patients suffered from polyneuropathy, three had liver enzyme abnormalities.

Of all 118 patients, 85 had no underlying disease or any other medical problems. Eight patients had diabetes mellitus, one patient had a malignancy. Five patients had other associated medical problems such as smoking or alcohol abuse. Two patients died of disseminated tuberculosis. Two patients were on immunosuppressive therapy.15 patients were on HAART therapy. Tuberculosis is seen when there is weakening of the immune system in conditions like HIV/AIDS, malnutrition, diabetes, prolonged treatment with steroids, immunosuppressive therapy, chronic renal failure and gastrectomy.

CONCLUSIONS

Isolation of mycobacteria from clinical samples by culture still represents the corner stone on which definitive diagnosis of tuberculosis. Mycobacterial culture provides the necessary isolates for conventional drug susceptibility test, and species identification.

REFERENCES

1. World Health Organization. Global tuberculosis report 2018. Geneva, Switzerland: World Health Organization;2018.https://www.who.int/ tb/publications/ global_ report/en/
2. E. Kulchavenya, "Extrapulmonary tuberculosis: Are statistical reports accurate?" Therapeutic Advances in Infectious Disease, vol.2,no.2,pp.61–70,2014.
3. Purohit M, Mustafa T. Laboratory Diagnosis of Extra-pulmonary Tuberculosis (EPTB) in Resource-constrained Setting: State of the Art, Challenges and the Need. J Clin Diagn Res. 2015;9(4):EE01–EE6. doi:10.7860/JCDR/2015/12422.5792
4. Davies PD, Pai M. The diagnosis and misdiagnosis of tuberculosis. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease. 2008;12(11):1226-34.
5. Revised National TB Control Programme, Training Manual for Mycobacterium tuberculosis Culture and Drug susceptibility testing, Central TB Division, Ministry of Health and Family Welfare, New Delhi, India, 2009 April.
6. Díaz-Infantes MS, Ruiz-Serrano MJ, Martínez-Sánchez L, Ortega A, Bouza E. Evaluation of the MB/BacT mycobacterium detection system for susceptibility testing of Mycobacterium tuberculosis. J Clin Microbiol. 2000;38(5):1988–1989
7. Pang Y, An J, Shu W, Huo F, Chu N, Gao M, et al. Epidemiology of Extrapulmonary Tuberculosis among Inpatients, China, 2008–2017. Emerg Infect Dis. 2019;25(3):457-464. https://dx.doi.org/10.3201/eid2503.180572
8. Chakravorthy S, Kamal M, Senand Jaya Sivaswami Tyagi. Diagnosis of extrapulmonary tuberculosis by smear, culture and PCR using universal sample processing technology Journal of clinical Microbiology. 2005; 4357-4362.
9. Palacios-Marmolejo A, Luna-Ramírez G, Ornelas-Perea M, et al. Diagnosis of extra pulmonary tuberculosis by culture. J Lung Pulm Respir Res. 2018;5(5):158–162. DOI: 10.15406/jlprr.2018.05.00184
10. S Rishi, P Sinha, B Malhotra, N Pal .A Comparative study for detection of mycobacteria by BACTEC MGIT 960, Lowenstein Jensen media and direct AFB smear examination IJMM. 2007 25(4): 383-6.
11. Orvankundil S, Jose BP, Yacoob FL, Sreenivasan S. Culture positivity of smear negative pulmonary and extrapulmonary tuberculosis- A study from North Kerala, India. J Family Med Prim Care 2019;8:2903-7
12. Conception F. Ang, Myrna T Mendoza, Wilma C Bulatao, Marc Agnew M. Culture isolation of Mycobacteria by MB/BacT system compared to Lowenstein Jensen Egg medium. Phil J Microbiol Infect Dis 2001; 30(2): 40-43.
13. B Mahadev, N Srikantarammu, P James, PG Mathew et al. Comparison between rapid colorimetric Mycobacterial isolation and susceptibility testing method using LJ medium. Indian J. Tub 2001; 48: 129
14. Gebremariam G, Asmamaw G, Hussen M, Hailemariam MZ, Asegu D, Astatkie A, et al. Impact of HIV Status on Treatment Outcome of Tuberculosis Patients Registered at Arsi Negele Health Center, Southern Ethiopia: A Six Year Retrospective Study. PloS One. 2016;11(4):e0153239
15. Ayyagiri et.al. Spectrum of opportunistic infections in HIV infected cases in a tertiary care hospital. Ind J Tuber. 1999; 17(2): 78.
16. BK Dash, SR Panigrahi. Extra-pulmonary lesions among HIV positive tuberculosis individuals and response to chemotherapy. Ind J Tub. 2000; 47: 245.
17. R. Shivakoti, D. Sharma, G. Mamoon, and K. Pham, "Association of HIV infection with extrapulmonary tuberculosis: a systematicreview," Infection, vol.45, no.1, pp. 11–21,2017.
18. Tanyalak Parimon et al Unexpected Pulmonary Involvement in Extrapulmonary Tuberculosis Patients. Chest September, 2008; 134: 3. www.chestjournal.org