



Isolation and Identification of Mycobacterium from Pus Samples from Various Lesions

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ABSTRACT

Aim & objective: To study the frequency of isolation of Mycobacterium the pus (purulent discharge) samples collected from different lesions. *Materials & methods:* The study was carried in the department of Microbiology, during July 2009-June 2010. Total 120 specimens of clinically suspected patients were submitted in the microbiology laboratory such as pus from lymph node aspirate, non healing ulcer, postoperative wound discharge, abscesses from various site. Lowenstein-Jensen media was used for culture. *Results :* 16.32% samples were positive for mycobacteria. Mycobacterium tuberculosis was isolated from 13.26% samples. While 3.06% were isolates were atypical mycobacteria. Male and female ratio was almost equal among positive cases. *Conclusion:* The results suggest that, emphasis should be placed on laboratory diagnosis and treatment of extra-pulmonary tuberculosis.

Keywords : Tuberculosis, extrapulmonary, Laboratory Techniques and, Atypical mycobacteria

Introduction :

The most common site of tuberculosis remains the lungs while amongst the extrapulmonary sites lymph node affecting mostly the cervical group of lymph nodes are the common site. Other forms of extrapulmonary tuberculosis are tuberculous pleural effusion, tuberculous pericardial effusion, intestinal, genitourinary, meningeal, bone, skin, and breast tuberculosis¹. Even then, it is difficult to isolate Mycobacterium tuberculosis due the small number of organisms present at these sites.² Various international studies in the world have focused on the problem of extra-pulmonary tuberculosis including pus from reporting a high frequency.³⁻⁷ It is estimated that ~40% of the population in the country is infected with the TB bacilli and it is estimated that about 10% of them will develop TB disease during their lifetime.⁸ The study had focused to study the frequency of isolation of Mycobacterium species from the pus samples collected from the clinically suspected various lesions .

Materials and Method

This study was conducted for detection of tuberculosis infection for the period of July 2009 to June 2010. It is observed that a total of 120 specimens were analyzed during the said period which included lymph node aspirate, pus from patients of Cold abscess chronic discharging ,sinus, aspirate from lymph node, the specimens which were submitted for routine culture and those were negative were also processed for mycobacteriology. Samples from soft tissue abscess at different sites, namely the gluteus ,breast, mastoid abscess and inguinal area, lymph node aspirate were collected. Specimens from Pott's spine, non healing ulcer, pus from osteomyelitis, osteoarthritis an also processed for mycobacteriology. Biopsy and pus specimens were obtained from patients with lymphadenitis, abscesses or sinuses of chronic or recurrent nature Specimens were collected under all aseptic precautions & submitted in sterile containers in microbiology department

Pus (various tissues, purulent fluids and aspirates, wound discharge) from the clinically suspected patients were processed in the microbiology department. Smears were prepared and stained with Zeil Neelsen method .After appropriate preparation the material were cultured in Lowenstein Jensen (L-J) medium .Culture was done on 2 slopes of Lowenstein-Jensen (LJ) medium and incubated at 37°C and checked for growth daily for one week and then twice a week for 10 weeks after which they were discarded if no growth was seen.

The colour and colony characteristic of growth on LJ medium were recorded. Three tests were performed and on the basis of these mycobacteria were broadly classified in to M. tuberculosis and non-tuberculous mycobacteria. Niacin test, Thermolabile Catalase Test, Growth on para-nitrobenzoic acid (PNB) incorporated in LJ media:

The isolates were further subjected to the tests in order to separate out the Mycobacterium tuberculosis from Non-Tuberculous Mycobacteria (NTM). All the tests performed for differentiation and identification were as per TRC manual 1987, Tuberculosis Bacteriology- Organisation and Practice (Collins, Grange and Yates 1997),

The strains that were Niacin positive, catalase negative, not showed growth on PNB media were identified as M.tuberculosis. Niacin negative, catalase positive and showed growths on PNB media were considered as NTM. .

Results:

Out of the 120 cases analyzed from clinical suspected patients. Lymph nodes fluid and pus collected from various lesions like Pott,s spine , cold abscess at various site were sent for analysis (table 1). Pus out of which 19(15.8%) turned out to be positive for mycobacteria. Three samples including specimen from non healing ulcer of 6 months duration at ax-

illa from female patient ,pus from male patient having breast abscess & male patients having abscess from cervical lymph node were smear positive but not grown on LJ medium. For three specimens no acid fast bacillus was seen but on culture there was growth of M.tuberculosis on LJ medium. 16 samples were culture positive amongst which 3 identified as atypical mycobacteria & 13identified as M.tuberculosis grown on LJ medium. Out of the 120 samples positive yield was found in 16 samples (13.33%) of which 3(2.5%) were identified as non tuberculous mycobacteria. (Table 2)

Table.1-Clinical Samples included in the study

No.of positive samples	Specimens	No of positive samples
Wound discharge/non healing ulcered	48	2
Aspirate from Abscess	42	5
Lymph node aspirate	30	12

Table :2 positivity of the samples by smear & culture

TOTAL POSITIVE	Smear positive Culture positive specimen		Smear positive Culture negative	Smear negative culture positive
	M.tuberculosis	NTM		
19	16	3	3	3

Discussion:

Definitive diagnosis of extrapulmonary tuberculosis on the basis of clinical examination is difficult as not having the typical presentation and non specific characteristics. 1 it is often missed or misdiagnosed so the first step in its diagnosis is its awareness and a high index of suspicion by the physicians. Laboratory aid is commonly required to come to a conclusion. But question remained regarding the out come of these laboratory investigations. Direct visualization through Ziel Neelsen (ZN) stain is commonly missed in single or paucibacillary specimen due to the very few number of AFB. A definitive diagnosis therefore remains solely in the hand of a culture growth Microscopic examination and culture of specimens

are necessary for definitive diagnosis.2 The gold standard is culture or direct visualization of mycobacteria. The major problem is the very low yield of mycobacteria in extrapulmonary samples resulting in a low sensitivity of Acid Fast Bacilli (AFB) smear and culture. Direct visualization through Ziehl Neelsen (ZN) stain is commonly missed in single or paucibacillary specimen due to the very few number of AFB. A definitive diagnosis therefore remains solely in the hand of a culture growth9. Mahdev et al (2001)10and Butt et (2003)2al in India found 23.5% and 21.3% isolates respectively in their samples which were similar to this study, showing an almost equal rate of involvement among the sexes. Similar results have been observed before by Hussain et al (2004)11 . The samples most commonly sent for analysis were lymph node aspirates Out of the 120 samples sent, 16 were positive. Lymphnode biopsy or aspirate showed the highest positive yield in culture which was also observed by Butt et al(2003)2 in the study of extrapulmonary samples for tuberculosis.Butt et al observed 0.8% isolates from pus were NTM in our study ,2.5% isolates were NTM which affects the management of the patients as are resistant to antitubercular drugs.Three samples including specimen from non healing ulcer of 6 months duration at axilla from female patient ,pus from male patient having breast abscess & male patients having abscess from cervical lymph node were smear positive but not grown on LJ medium.

Conclusion

Finally it can be said that the most of the infections which might be negative by routine aerobic crulture may be positive for acid fast bacilli .It is helpful to culture these specimen for mycobacteriology as sensitivity for culture is more than staining and to identify these organisms is helpful for management as NTM are resistant to antitubercle drugs. The drugs currently used as first line of treatment are well in action giving a glimpse of a bright future in the field of TB control.

Conflict of interest: None

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