Original Article

Use of Mycobacterial Culture for the Diagnosis of Smear Negative TB Cases Among New Outpatients at NIDCH, Dhaka

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Abstract

In this prospective study out of 300 randomly selected clinically and/or radiologically suspected TB patients ultimately data of 277 cases were analyzed. Among 23 excluded cases, 6 were positive by microscopy, 5 responded with broad spectrum antibiotics, and 12 were contaminated. After 2 months follow up with anti TB drugs, a total of 124 TB cases were diagnosed of which 121 (44%)were culture positive. Three cases were diagnosed by empirical anti TB treatment. A total of 153 cases were true negative by mycobacterial culture. Of 121 isolates 119 were typical and 2 were atypical mycobacteria. In this group, the sensitivity, specificity, positive predictive value and negative predictive value of mycobacterial culture were 97.63%, 100%, 100% and 98.1% respectively. Among the culture proven cases; 79% were prevalent in male and 21% in female. Case positivity was significantly higher among patient having symptoms of cough with hemophtysis with or without fever(58%) than the patients with constitutional symptoms. No advanced or cavitary lesions were seen on X-ray among culture proven cases. Majority culture proven cases(72%) had minimal lesion on X-ray. After two months anti TB therapy 119 culture proven cases and three culture negative cases improved clinically and radiologically.

Introduction:

National Institute of Diseases of Chest and Hospital(NIDCH), Bangladesh is a tertiary level hospital specially designed to treat difficult TB cases like smear negative cases, MDR-TB cases, chronic and relapse cases and also extra pulmonary TB cases. 16% of TB cases are new smear negative cases[2]. A good number of these patients are being referred to NIDCH from all over Bangladesh for better management. Traditionally, they are empirically treated with anti TB drugs after having a presumptive diagnosis with ancient practice of positive X-ray and Mantoux test (MT) finding and high ESR. Although a good number of patients of this group are not really TB cases[5]. Besides technical errors of smear negativity, many medical conditions like

resistant bacterial infections, viral infections, carcinoma, lymphoma, asthma, chronic obstructive lung diseases(COPD) other allergic condition came to present as smear negative TB cases[6]. HIV infection also responsible to produce higher number of smear negative TB cases[7,8]. In a high burden country like Bangladesh , microscopy remains as gold standard but mycobacterial culture is superior to microscopy. It can detect cases with bacterial load up to 10 to 100 viable bacilli/ ml of sputum whereas microscopy can do it up to 5000 to 10000 bacilli/ml. Moreover microscopy can not detect viability, identity and anti TB drugs susceptibility   of TB bacilli[9,10,11]. For bacteriological evidence of smear negative TB cases culture is the next option to microscopy[6]. Unlike developed countries , the infectivity and mortality of smear negative TB cases is the same as that of smear positive TB cases in high burden countries[12]. Moreover , empirical TB treatment may give rise to chronic and MDR cases. Gradual expansion of culture laboratories and introduction of molecular diagnostics can help proper management of TB cases. In spite of high prevalence of smear negative TB cases there are only two review articles regarding this subject in

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last 20 years worldwide[6]. Until now there is no such study in Bangladesh. Therefore, this study will describe the case positivity and natural history of new smear negative TB cases by mycobacterial culture.

Matrials and Methods:

A total of 300 randomly selected patients having clinical and/or radiological features of tuberculosis attending out patients department of NIDCH were enrolled in this study during May to October-2008. Smear positive cases or the cases taking prior anti-TB drugs were excluded from the study. One spot sputum specimen (about 5 ml)and another morning specimen were collected under the guidance of a doctor. Both the specimens were sent to laboratory as soon as possible. Two direct smears were prepared from each of the two specimens for staining – one by Ziehl-Nelsen(Z-N) and the other by Flurochrome(FM) techniques and were examined under microscope following standard operating procedure (SOP). If any cases found positive, then it was excluded from the study.

The remaining part of each specimen was processed for culture by digestion, decontamination and concentration following modified Petroff's method and were inoculated on to two slopes of Lowenstein- Jensen(L-J) media for six weeks. Readings were taken every week for six weeks. The identity of the isolates were made by growth rate, colony morphology, P-nitrobenzoic acid(PNB)susceptibility, catalase and nitrate reduction tests. Drugs susceptibility tests were not performed.

Later on, all the cases were given a course of broad spectrum antibiotic(Levofloxacin/ciprofloxacin for one week) and were asked to perform a fresh chest X-ray after two weeks. If the patients improved clinically and radiologically they were excluded from the study. Rest of the patients were given anti TB drugs following the national anti – TB treatment guidelines . After two months , clinical and radiological improvement were noted. HIV screening was not performed.

Results:

Among 121 culture proven cases 79% were male and 21% were female. The culture positivity were more prevalent in the age group 41 years or above (Table I)

Table I: Age sex distribution of culture positive TB cases among actual study population

Age Group	Male n =201(72.6%) Culture		Female n =76(27.4%) Culture	
	Positive	Negative	Positive	Negative
10-20 yrs. n= 4+6=10	0	04	02(33.9%)	04(67%)
21-30 yrs. n=67+32=99	28 (42%)	39 (58%)	11 (34%)	21 (66%)
31-40 yrs. n=36+16=52	16 (44%)	20 (56%)	06 (37%)	10 (63%)
41-50 yrs n=37+16=53	22 (59%)	15 (41%)	05 (25%)	12 (75%)
>50 yrs. n= 57+6=63	30 (52%)	27 (48%)	2 (33%)	4 (67%)
Total n= 277	96(79%)	105	25(21%)	51
Total culture positive cases: 121 (44%)				

Out of 300 patients,   6 were smear positive,5 responded with broad spectrum antibiotics and 12 were contaminated and all three groups were excluded .   277 (92.3%) patients comprised the actual study population (Table II) .

Table II : Culture Results

Characters	Number	%
Total study population	300	100
Case excluded		
a) smear positive	06*	2.0
b) antibiotic responder	05	1.7
c) contamination	12	4.0
Total	23	7.7
Actual study population	277	92.3

*2 by only FM, 4 by both FM & Z-N methods

121 (44%) of the remaining 277 were culture positive and the rest of the 156 patients were culture negative cases (table III).

Table III : Distribution of Culture positive and negative cases among the actual study population

Actual study population (n=277)	Total number	Percentage
Culture positive	121	44 %
Culture negative	56	56 %

In addition to 121 culture positive cases , 3 patients of the 156 culture negative cases   improved by anti TB drugs and the

remaining 153 were considered as true negative cases (Table IV) .

Table IV : Distribution of cases considered as TB

	Total No	Percentage
Number of study subjects that were culture positive	121	97.6
Number of study subjects that were culture negative but improved with anti TB treatment	03	2.4
Total number of TB cases	124	100

Among 121 isolates , 119 were typical mycobacteria and 2 atypical mycobacteria (Table V).

Table V: Identity of Isolates

Identity	No	Percentage
Mycobacterium tuberculosis	19	99.30
Atypical Mycobacteria	02	0.7
Total	121	100

Of the 121 culture positive cases, 27 presented with only cough, 49 cough with low grade fever, 24 cough with weight loss, 14 cough, hemoptysis with or without fever only 7 with cough and constitutional symptoms (Table VI)

Table VI : Relation of Clinical Finding and Culture Positive Cases

Clinical findings*	Total patients no (%)	Culture Positive No	% of total case
Only Cough	69(25%)	27	39
Cough with low Grade fever	92(33%)	49	48
Cough with weight loss	56(20%)	24	43
Cough, hemophtysis with or without fever	23(08%)	14	61
Cough with constitutional symptoms	37(13%)	07	19
Total	277(100%)	121	44

*May or may not associated with X-ray findings

There were no advanced or cavitary lesions in X-ray of any patients. However 113 cases had minimal diseases, 6 had moderate non cavitary lesion but only 02 culture positive cases had no shadow on the X-ray (Table VII) .

Table VII : Relation of X-ray finding and Culture positive cases

X-ray finding	Total no of patients No	%	Culture Positive No	% of total patients
No shadow	112	40	02	1.8
Minimal disease	158	57	113	72
Moderate non- cavitary	97	2.5	06	86
Advanced cavitary	0	0	0	0
Total	277	100	121	44

When, culture results were compared to total TB cases diagnosed, the sensitivity , specificity, positive predictive value and negative predictive value were 97.63%,100%,100% and 98% respectively(Table VIII).

Table VIII : Comparison of culture result among total TB cases

Culture Result	TB cases Positive	Negative	Total
Positive	121	0	121
Negative	03	153	156
Total	124	153	277

Sensitivity = 97.63% Specificity = 100%

Positive predictive value (PPV)= 100%   Negative predictive value = 98.1%

Discussion:

In this study the case positivity rate was 44% (121 of 277). The remaining 54% cases were may be due to resistant bacterial infections , viral infection . parasitic infestations,lymphoma, carcinoma, allergic lung conditions and asthma or COPD[6]. Some of them may be due to tuberculosis but at present they are not detectable by culture. The empirical anti TB treatment of negative cases would be over-diagnosed. But culture can eliminate these clinical

problem in TB case management. There are variable reports regarding culture positivity among smear negative cases. In one review study (Van Deun A.,2004) showed 24 to 62% positivity rate among different geographical locations[3]. Other natural history of smear negative had similarities with smear positive TB cases[2].

Conclusion:

Mycobacterial culture appears as good tool for diagnosis of smear negative TB cases specially in TB/HIV co infections. It can eliminate the problem of over diagnosis of smear negative TB cases with traditional clinical and radiological basis. NTP Bangladesh can adopt policy for case detection among smear negative cases by culture through expanding culture facilities all over the country.

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