

Re-evaluation of Smear Negative Suspected Tuberculosis Patients by GeneXpert

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Abstract

Introduction: Tuberculosis (TB) is one of the major public health problems. Sputum microscopy even today is considered as most important laboratory diagnosis of TB though its negative result does not exclude tuberculosis.

Objective: To evaluate the smear negative suspected TB patients by GeneXpert.

Materials and Methods: This descriptive cross-sectional study was conducted in Surya Kanto Hospital, Mymensingh from September 2014 to September 2017. Total 300 suspected TB patients were selected and sputum microscopy was performed then re-evaluation of the smear negative cases was carried out by GeneXpert.

Results: The mean age of the patients was 38.87±13.78 years with a male to female ratio 2.1: 1 and 86% was in economically productive age of life. About 13.1% smear negative suspects were diagnosed as TB positive by GeneXpert.

Conclusion: GeneXpert played effective role to re-evaluate the smear negative TB cases. So, GeneXpert may be recommended routinely for TB detection.

Key-words: Tuberculosis, Smear negative, Re-evaluation, GeneXpert.

Introduction

In Bangladesh, tuberculosis (TB) is one of the major public health problems. In 2016 World Health Organization (WHO) rank Bangladesh as 7th among the world's 22 high-burden TB countries^{1,2}. With an estimated population of 161 million, these estimates correspond with an annual incidence of about 362,000 all forms of TB cases and about 73,000 deaths annually². Mortality rate globally range from 1.6 to 2.2 million lives per year³. Sputum microscopy still remains the primary tool for the laboratory diagnosis of TB. National TB Control Program (NTP) has successfully treated 100254 (92%) of the 109012 new smear positive cases registered in 2009⁴.

In 2016, Damien Foundation registered 10787 smear negative Pulmonary TB clinically diagnosed and extra pulmonary TB². Smear positive TB is responsible for spreading the diseases in the community. Delay in the diagnosis worsen disease process, causes risk of death and enhance TB transmission. In many countries, good quality microscopy of sputum specimens identifies the vast majority (95-98%) of smear positive TB patients⁵. In Jobayer M, among 125 smear negative samples 13(10.4%) were positive by culture and 21(16.8%) were positive by PCR⁶.

Shweta et al⁷ found that co-infection of visceral leishmaniasis and pulmonary tuberculosis are increasing public health problem especially in developing country. This concurrency may enhance increasing tuberculosis in humans. In 2006 WHO stated despite the considerable progress of TB; 53% detection rate in 2004 and 82% cure rate in 2003 acceleration is required to achieve the targets of 2015⁶. More than 81% of TB cases and deaths comes from developing countries; the TB situations in those countries is aggravated by high prevalence of HIV, drug resistance, social inequalities, poor TB control efforts and inadequate health care spending⁷. National strategic plan of NTP covering the period 2015-2020 contains strategies and interventions based on the principles outlined the WHO's "End TB Strategy" that would enable the NTP to achieve the End TB Strategy's milestones by 2025^{3,8}.

Diagnosis of TB is mainly based on microscopic detection of acid fast bacilli in smear which has 40 to 70% sensitivity and culture which needs up to 8 weeks time⁹. Smear negative has its hidden diagnostic significance, so is as important as smear positive. Negative sputum smear does not exclude TB in highly suspected cases¹⁰. GeneXpert can identify presence of Mycobacterium in smear negative sputum as well. It can be a useful diagnostic method in patients of suspected pulmonary tuberculosis; especially in the smear negative cases¹¹. This study was intended to re-evaluation of smear negative suspected TB patients by GeneXpert reported to Surya Kanto Hospital, Mymensingh.

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Materials and Methods

This was a descriptive cross-sectional study conducted within the period of September 2014 to September 2017 at the Surya Kanto Hospital, Mymensingh and laboratory tests were done at the DOTS corner of Mymensingh Medical College (MMC) Hospital and Mymensingh TB and Leprosy Control Project (MTLCP) Shambhuganj, as part of the total research to see the smear microscopy of TB. The study was designed in such a way that for all suspected tuberculosis patients, confirmation of TB was first concern with sputum microscopy and then further evaluation of the smear negative cases with GeneXpert. Two sputum smears for each patient were done and examined under microscope following National Guidelines and Operation Manual for TB control 2013¹². Sputum of morning of reporting day and sputum after reporting to DOTS corner (spot sample) were collected and the smear was prepared as 1st and 2nd respectively. A total of 300 highly suspected patients of pulmonary TB from Surya Kanto Hospital both admitted (180) and at outdoor (120) from Trishal, Fulbaria, Madhupur and Chatmohor Upozilla areas who submitted their sputum were included. After sputum microscopy; sputum negative cases were sent to MTLCP for GeneXpert to see if there were any Mycobacterium positive cases. Data were compiled and analyzed by using SPSS version 20.0. The study was ethically cleared and approved by ethical review committee of MMC. Sputum obtained from patients were prepared for fluorescence microscopy by auramine staining method using 0.1% auramine, 0.5% acid-alcohol as de-colouriser and 0.3% methylene blue or 0.5% potassium permanganate as counter stain as per the hand book of Stop TB Partnership, Global edi 2013¹³.

Results

Among the study population 204 (68.0%) were male and 96(32.0%) were female. The male: female ratio of TB suspects was 2.1: 1 and the mean age was 38.87 ± 13.78 years. Maximum 86% of patients were found within 16-60 years of age (Table-I).

Table-I: Distribution patients according to age and sex (n=300)

Age group (in years)	Sex of patients		Total Frequency (%)
	Male Frequency (%)	Female Frequency (%)	
≤ 15	12 (4.0)	6 (2.0)	18 (6)
16 to 30	52 (17.3)	26 (8.7)	78 (26.0)
31 to 45	68 (22.7)	35 (11.7)	103 (34.3)
46 to 60	56 (18.7)	22 (7.3)	78 (26.0)
61 to 75	10 (3.3)	5 (1.7)	15 (5.0)
≥76	6 (2.0)	2 (0.7)	8 (2.7)
Total	204 (68)	96 (32)	300 (100)

Considering the monthly income of family of suspects it was found that majority 216(72.0%) of the patients were from lower economic status whose monthly family income were within 10,000 Taka (\$118) per month (Table-II).

Table-II: Distribution of respondents by monthly family income and diagnosis (n=300)

Monthly family income (in Taka)	Diagnosis		Total Frequency (%)
	TB negative Frequency (%)	TB positive Frequency (%)	
≤ 5000	50 (16.7)	84 (28.0)	134 (44.7)
5001 to 10,000	30 (10)	52 (17.3)	82 (27.3)
10001 to 20,000	23 (7.7)	21 (7.0)	44 (14.7)
20001 to 30,000	10 (3.3)	16 (5.3)	26 (8.7)
≥ 30,001	07 (2.3)	07 (2.3)	14 (4.7)
Total	120 (40.0)	180 (60.0)	300 (100)

$$\chi^2 = 4.172; df = 4; p > 0.05$$

Out of 300 suspected TB patients; by smear microscopy 162 (54%) were found smear positive and rest were smear negative (Table-III).

Table-III: Smear Microscopy Result of Suspected TB Patient (n=300)

Smear Microscopy Result	Frequency	%
Positive	162	54
Negative	138	46
Total	300	100

All the smear negative specimen were sent for GeneXpert and out of 138 cases 18 (13.04%) were found TB positive and rest were negative (Table-IV).

Table-IV: GeneXpert result of smear negative cases (n=138)

GeneXpert Result	Frequency	Percentage
Positive	18	13.1
Negative	120	86.9
Total	138	100

By smear microscopy out of 300 suspected TB patients; 162 cases were diagnosed as TB positive and all 138 smear negative specimen were re-evaluated by GeneXpert and 18 were diagnosed as TB positive. Finally among 300 total patients 180 were diagnosed as TB positive and rest were negative (Table-V).

Table-V: Distribution of Respondents by diagnosis (n=300)

Diagnosis	Sputum Smear Microscopy		Total Frequency (%)
	Smear positive Frequency (%)	Smear negative Frequency (%)	
TB positive	162 (54)	18 (6)*	180 (60)
TB negative	00	120 (40)	120 (40)
Total	162 (54)	138 (46)	300 (100)

*18 smear negative cases were diagnosed as TB positive by GeneXpert.

Discussion

In this study the mean age was found 38.87 ± 13.78 years, the male female ratio was found 2.1:1. In the study of Jermiah Seni et al the mean age was 36.1 and male female ratio was 1.17:1 may be it was due to higher HIV positivity rate where male female equally affected and lesser elderly population¹⁴. In the study Takele Tedesser et al the mean age was found 34 years and male female ratio was 0.9:1 where it might be due to two thirds of the symptomatic sputum smear positive tuberculosis remains undiagnosed and higher HIV prevalence rate four to six times¹⁵. In the present study majority TB suspects and positive cases were found in productive year of life 16-60 years. Karmaker H et al in their study mentioned the age group 16 to 45 as most vulnerable for TB and consisting 78.7% of total study Population¹⁶.

In this study, smear positive cases were found in 54.0% of all suspects with the male female ratio of 2.1:1. This number was much higher as the study included only TB diagnosed indoor and highly TB suspected outdoor patients and it was much higher to the recommended rate 10% of IUATLD¹⁷ which is a community calculation. Horie T et al in Hanoi, mentioned that in Vietnam the smear positive case finding was 3.3 times higher for females than males and was due to the active case finding via household visits that reduces the social inequity in accessing health services¹⁸. Yassin and Cuevass in their study found that the smear positivity rate (two or more positive smears) was 25%, with a range of 16.8-36.4% per zone. This exceeds the international recommendations of examining 10 suspects to identify one case¹⁹.

It is evident from this study about monthly family income of the respondents that majority 216(72.0%) were from lower socio-economic status. Their monthly family income was within Taka 10,000 (\$118), which is of course from poorer economic society and overall Mymensingh people has comparatively low monthly income and are prone to tuberculosis. Majority (61.0%) of the patients had monthly income less than 25,000 BDT were found in Irfan SD which is much higher than that of this study. The study Irfan SD was conducted at Shyamoli area of Dhaka city and obviously the monthly income supposedly higher and respondents were less susceptible to tuberculosis²⁰.

In this study, total smear positive cases were found 162 in 24 months of patients' investigation. From Damien Foundation Mymensingh it was found that in 2008 new positive cases were 254, in 2009 it was 328, in 2010 was 294, in 2011 was 300 and in 2012 it was 215 cases². This study finding 162 are satisfying similarly with the fore-going. It depicts that the tuberculosis is still maintaining same pattern as it was before six years. Uniqueness of this study is that smear negative 138

cases which were re-evaluated further and 18 cases were detected as positive. Diagnostic difficulties arise when sputum smears are negative for acid-fast bacilli in tuberculin-positive patients with compatible symptoms and chest radiographs for tuberculosis. Many of these smear-negative patients yield positive cultures for TB whereas others remain culturally negative. Several studies have shown that many smear-negative/culture-negative patients will develop bacteriologically positive disease later. Often, physicians are unable to decide whether to initiate chemotherapy or to wait for the culture results²¹. In a study Jobayer M et al stated that among 125 smear negative samples 13 yielded growth in culture and 21 samples were found positive by PCR²². As such smear negative samples do not mean the absence of tuberculosis and warrants re-examination. This study tried to delineate that amongst smear negative suspects there may have positive cases which remain undiagnosed, lack treatments and cause threat to the community.

In this study out of 138 sputum negative TB suspects 18 (13.1%) cases were identified as TB positive by GeneXpert and is higher than national 3.6 (Figure-1). In Aurin T H et al among 300 samples, 193(64.3%) cases were found to be positive by GeneXpert methods²³. Despite success in tuberculosis control, multi-drug resistant tuberculosis in Bangladesh is increasing and currently multi-drug-resistant tuberculosis rate is 3.6% in new cases and 19% in re-treatment cases²⁴. Another study Lombardi G 2017, out of 234 smear-negative culture-positive samples, 137(58.5%) were respiratory and 97(41.5%) non-respiratory. Sensitivity of Xpert in smear-negative culture-confirmed TB was 73.1% (171/234):73.0% (100/137) for PTB²⁵. In Shrestha P et al it was found that 258 smear negative cases were re-evaluated by GeneXpert of which 55(21.3%) were positive for TB²⁶. In this study from each suspected patients two sputum samples were performed smear microscopy and found effective. WHO recommends the number of specimens to be examined for screening of TB cases can be reduced from three to two, in places where a well-functioning external quality assurance system exists, where the workload is very high and human resources are limited (WHO policy 2007)²⁷.

Conclusion

Smear positive patients are taken under treatment, and people associated can observe preventive means as well. But in smear negative cases, patient feels smart that he or she is free of tuberculosis. These patients are not properly taken care. In this study, a substantial number of cases became negative, obviously there were some unearthen positive cases that needed re-evaluation. As such, GeneXpert played effective role to re-evaluate the smear negative cases where it successfully detected positive cases and brought those patients under treatment.

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References

1. Global Tuberculosis report 2016.
2. Damien foundation Bangladesh annual report 2016.
3. Rahman F, Munshi SK, Kamal SMM et al. Comparison of different microscopic methods with conventional TB culture. *Stam J Microbiol* 2011; 1:46-50.
4. National Tuberculosis Control Programme (NTP): Achieved and Yet to Achieve. *Birdem Med J* 2013; 3(1):1-3.
5. WHO. Same day diagnosis of tuberculosis by microscopy 2011. WHO/HTM/TB/2011.7
6. WHO 2006 Tuberculosis facts, Stop TB Partnership.
7. Dye C, Williams B. The population dynamics and control of tuberculosis. *Science* 2010; 328:856-61.
8. Tuberculosis control in Bangladesh, Annual Report 2017, NTP.
9. Runa F, Yasmin M, Hoq MM et al. Molecular versus conventional methods: Clinical evaluation of different methods for the diagnosis of tuberculosis in Bangladesh. *J Microbiol Immunol Infect* 2011; 44(2):101-5.
10. Brodie D, Schluger NW. The diagnosis of Tuberculosis. *Clin Chest Med* 2005; 26:247-71.
11. Ahmed MT. Comparison of Xpert MTB/RIF Assay with Smear Microscopy and Culture for the detection of Pulmonary Tuberculosis. Dissertation submitted to Dept of Mathematics and Natural Sciences. BRAC University; Oct 2017.
12. National Guidelines and Operation Manual for Tuberculosis control (NTP), 5th ed, 2013.
13. The hand book, Global edition, Stop TB Partnership 2013- Laboratory Diagnosis of Tuberculosis by Sputum Microscopy.
14. Seni J, Kidenya BR, Obassy E et al. Low sputum smear positive tuberculosis among pulmonary tuberculosis suspects in a tertiary hospital in Mwanza, Tanzania. *Tanzan J Health Res* 2012; 14(2):115-20.
15. Tadesse T, Demissie M, Berhane Y et al. Two-thirds of smear-positive tuberculosis cases in the community were undiagnosed in Northwest Ethiopia: Population based cross sectional study. *PLoS One* 2011; 6(12):e28258.
16. Karmaker H, Basar MA, Karim MR et al. An Epidemiological Study of Drug Resistant Tuberculosis Cases: Survey in the Northern Part of Bangladesh. *Public Health Research* 2016, 6(2):52-8.
17. International Union Against Tuberculosis and Liver Disease (IUATLD) 1998.
18. Horie T, Lien LT, Tuan LA et al. A survey of tuberculosis prevalence in Hanoi, Vietnam. *Int J Tuberc Lung Disease* 2007; 11:562-6.
19. Yassin MA and Cuevas LE. How many sputum smears are necessary for case finding in pulmonary tuberculosis? *Tropical Medicine and International Health* 2003; 8:927-32.
20. Irfan SD, Faruque MO, Islam MU et al. Socio-Demographic determinants of adult tuberculosis: A matched case-control study in Bangladesh. *American Journal of Infectious Diseases* 2017.
21. Dutt AK, Stead WW. Smear-negative pulmonary tuberculosis. *Semin Respir Infect* 1994; 9(2):113-9.
22. Jobayer M, Shamsuzzaman SM, Mamun ZK. Detection of Mycobacterium tuberculosis in smear negative sputum by PCR. *Bangladesh J Med Microbiol* 2012; 6(2):2-6.
23. Aurin TH, Munshi SK, Kamal SMM et al. Molecular Approaches for Detection of the Multi-Drug Resistant Tuberculosis (MDR-TB) in Bangladesh. *Plos One* 16 June 2014.
24. Lombardi G, Gregori VD, Girometti N et al. Diagnosis of smear-negative tuberculosis is greatly improved by Xpert MTB/RIF. *Plos One*; 21 April 2017. Available at <https://doi.org/10.1371/journal.pone.0176186>
25. WHO policy - TB diagnostics and laboratory strengthening, 2007.
26. Shrestha P, Arjyal A, Caws M et al. The Application of GeneXpert MTB/RIF for Smear-Negative TB Diagnosis as a Fee-Paying Service at a South Asian General Hospital. *Tuberculosis Research and Treatment* 2015, Article ID 102430, 6 pages. <http://dx.doi.org/10.1155/2015/102430>.
27. Saleem S, Shabbir I, Iqbal R et al. Value of three sputum smear microscopy in diagnosis of pulmonary tuberculosis. *Pak J Med Res* 2007; 46(2)-94-7.