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Research Article

PERIPHERAL BLOOD SAMPLE MAY NOT THE SPECIMEN OF CHOICE FOR THE DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* (MTB) BY GENEXPERT PCR ASSAY

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ABSTRACT

Tuberculosis (TB) remains as a major global health burden including Bangladesh. The detection of *Mycobacterium tuberculosis* (MTB) has been emphasized at the onset of the disease with utmost accuracy and is a global priority for TB control. TB infects and localized to almost all organs of the body and is characterized accordingly. Therefore, the detection of MTB is being performed from different types of tissues and body fluids. Nucleic acid amplification assay is increasingly used worldwide for the detection of both pulmonary TB and extra-pulmonary TB. The aim of this study was to evaluate the choice of specimen for detection of MTB by using GeneXpert polymerase chain reaction (PCR) assay, a qualitative Real Time PCR assay. From clinically suspected patients, a total of 5,900 samples were collected and were investigated at Ibn Sina Diagnostics and Imaging Center, Dhanmondi, Dhaka, Bangladesh from 2011 to 2020. A total of 881 (14.93%) samples were detected MTB positive by GeneXpert PCR assay. These positive samples included sputum 292 (20%), pus 78 (15.12%), ascetic fluid 27 (7.5%), pleural fluid 22 (4.15%), pericardial fluid (9%), lymph node aspiration 283 (65.81%), bone marrow 3 (2.38%), BAL 53 (26.10%), urine 8 (3.81%), CSF 47 (6.71%), tissue 36 (9%), tracheal tissue (5%), breast abscess 4 (10%), and wound swab 17 (13.08%), but none (0.0%) of the 655 peripheral blood samples detected MTB positive. Both pulmonary and extra-pulmonary samples showed positive result in different percentage for MTB infection whereas peripheral blood samples didn't show any positive result during the study period and the detection of MTB showed some difference in male and female of different age groups indicating the urgency of early and quick attention for timely management and control.

Key words: *Mycobacterium tuberculosis (MTB), gene Xpert PCR assay, Pulmonary tuberculosis (PTB), extra-pulmonary tuberculosis (EPTB), body fluids, peripheral blood*

Introduction

Tuberculosis (TB) is the most infectious communicable disease and is a major cause of illness and death from a single infectious agent (surpassing HIV/AIDS) (WHO 2020) ranked within the top 10 microbial infections worldwide. The presence of TB is reported worldwide among all age groups. Around 10 million TB infections were reported globally in 2019.TB burden in Bangladesh is being ranked within top 30 countries worldwide with annual occurrence of 362,000

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new tuberculosis cases, according to WHO Global TB report 2019. Annual loss of life due to tuberculosis was estimated about 73,000. The *Mycobacterium tuberculosis* (MTB) is the causative agent and is the most predominant (Butt *et al.*, 2001), and is the facultative intracellular parasite (Manganelli *et al.*, 1999). The aerosol caused by an infected person due to coughs, sneezes or laughs is a major vehicle of person-to-person infection. The pulmonary TB is the most usual and typical location but the infection of other organs extra pulmonary TB can also occur. Precise and early detection of MTB is very important to get timely management and cure. The conventional methods for MTB diagnosis are usually sputum smear microscopy, chest radio-graphic findings and culture studies (Soini *et al.*, 2001). Due to low level of MTB in samples and time-consuming conventional methods (Styrt *et al.*, 1997) are still a considerable challenge and proved to be inefficient for its early and quick diagnosis. Currently nucleic acid-based assays are well adopted and found to be suitable for the detection of MTB which shows high degree of efficiency and specificity (Soini *et al.*, 2001) in both cases of pulmonary and extra-pulmonary TB. The nucleic acid-based assays also have some problem to diagnose MTB because of their facultative nature. More common TB is observed to be pulmonary disease but because of asymptomatic and nonspecific symptoms, extra-pulmonary TB noted to be quite severe (Sanjay *et al.*, 1998).

The current research work was designed emphasizing to explore and determine the specimen of choice for the early and quick detection of both pulmonary and extra-pulmonary MTB by Gene Xpert PCR assay from a variety of clinical samples collected from different organs and sites such as brain, bones, abdomen, heart, lymphatic system, urinary tract and lungs are well reported to be infected and localized by MTB. Therefore, the pleural fluid, peripheral blood, CSF, ascetic fluid, urine, Broncho alveolar lavage (BAL), pericardial fluid, pus and bone marrow were selected as specimen samples for MTB detection.

Materials and Methods

Collection of Samples from Patient

A total of 5,900 clinical samples were collected at Ibn Sina Diagnostic Centers and hospitals, and its branches in the Dhaka City from January 2011 to December 2020 for the detection of MTB from patients with high clinical symptoms of pulmonary or extra-pulmonary TB. The samples were sputum (n=1460), Pus (n=516), ascetic fluid (n=360), pleural fluid (n=530), pericardial fluid (n=100), lymph node aspirate (n=430), bone marrow (n=126), BAL (n=203), blood (n=655), urine (n=210), CSF (n=700), tissue (n=400), tracheal tissue (n=40), breast abscess (n=40), and wound swab (n=130).

Sample Processing and Heat Inactivation

The samples were heated at 80°C for 10 min immediately after collection to kill MTB (Amin *et al.*, 2011). Pretreatment of sputum samples were performed in a special solution (2.5% *N*-acetyl-L-cysteine-NaOH solution) by using the standard preparation method for mycobacterial diagnosis and decontamination by Kirchner solution (Roberts *et al.*, 1991). All the samples were centrifuged at 3,000 ×g for 15 min and pellets were collected. The pellets were used for the detection of MTB by GeneXpert PCR assay.

Xpert MTB/RIF Assay

Samples were processed according to manufacturer's protocol (Kits: Xpert MTB/RIF, Cepheid, USA). Sample and reagent were added in a 1:2 ratio to unprocessed specimen in 15ml falcon

tubes and the tubes were incubated at room temperature for 15 min during which the tubes were manually agitated twice at certain intervals. Then the test cartridges were filled with 2 ml of the inactivated materials by sterile disposable pipettes. The cartridges were loaded into the Gene Xpert Real-time PCR Machine (Cepheid, USA) and performed the analysis as per manufacturer's instruction. The collection and interpretation of data from MTB/RIF tests was software based and not user dependent (Boehme *et al.*, 2010).

Results

Presence of MTB among Patient under Study

Table 1 shows the positive *Mycobacterium tuberculosis* (MTB) among the clinically suspected patients from different types of specimens. Out of the total 5,900 samples, 881 (14.93%) samples were found positive. These included, sputum found positive 292(20%) out of 1460 samples, pus 78(15.12%) out of 516, ascetic fluid 27 (7.5%) out of 360, pleural fluid 22 (4.15%) out of 530, pericardial fluid 9 (9%) out of 100, lymph node aspiration 283 (65.81%)out of 430, bone marrow 3 (2.38%) out of 126, BAL 53 (26.10%) out of 203, Peripheral blood 0 (0.0%) out of 655, urine 8 (3.81%) out of 210, CSF 47 (6.71%) out 700, tissue 36 (9%) out of 400, tracheal aspirate 2 (5%) out of 40, breast abscess 4 (10%) out of 40, and wound swab 17 (13.08%) out of 130.The percentage of positivity of MTB varies from specimen to specimen and peripheral blood samples showed no positive result during the study period.

Presence of MTB in Male and Female Patients

In the current study, total 5,900 of different types of samples collected from clinically suspected tuberculosis patients were analyzed to detect MTB, where 3,396 (57.55%) samples from male patients and 2,504 (42.44%) from female patients. The percentages of positivity of total sample had been presented in Fig. 1 with some differences.

Table 1. Positivity rate of MTB of different type of specimens (n=5,900).

Sl. No	Type of samples	Total samples received	Positive samples	Percentage (%)
1	Sputum	1460	292	20.00
2	Pus	516	78	15.12
3	Ascetic fluid	360	27	7.50
4	Pleural fluid	530	22	4.15
5	Pericardial fluid	100	9	9.00
6	Lymph node aspiration	430	283	65.81
7	Bone Marrow	126	3	2.38
8	BAL	203	53	26.10
9	Blood	655	-	-
10	Urine	210	8	3.81
11	CSF	700	47	6.71
12	Tissue	400	36	9.00
13	Tracheal Aspirate	40	2	5.00
14	Breast abscesses	40	4	10.00
15	Wound swab	130	17	18.08
Total		5,900	881	14.93

**Presence of MTB in male and female patients*

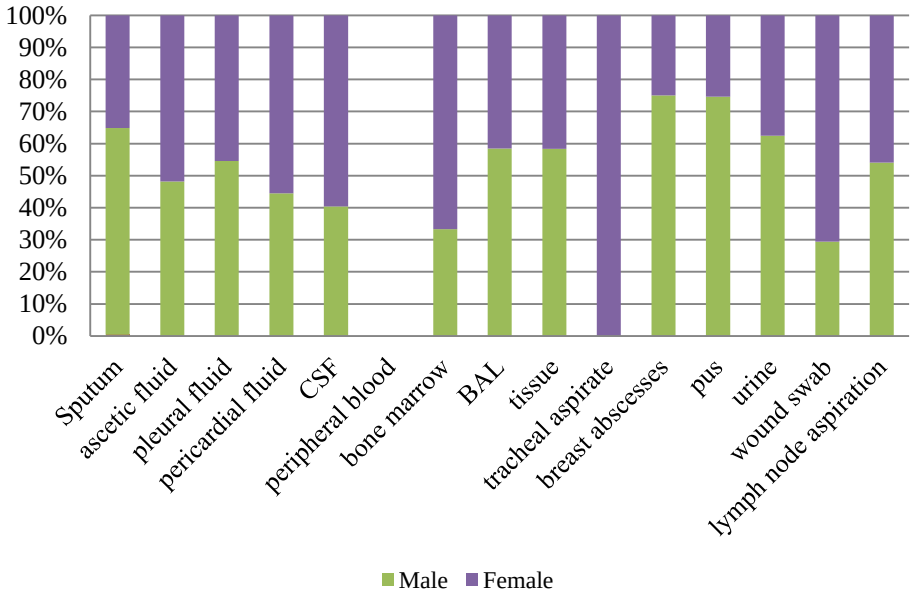
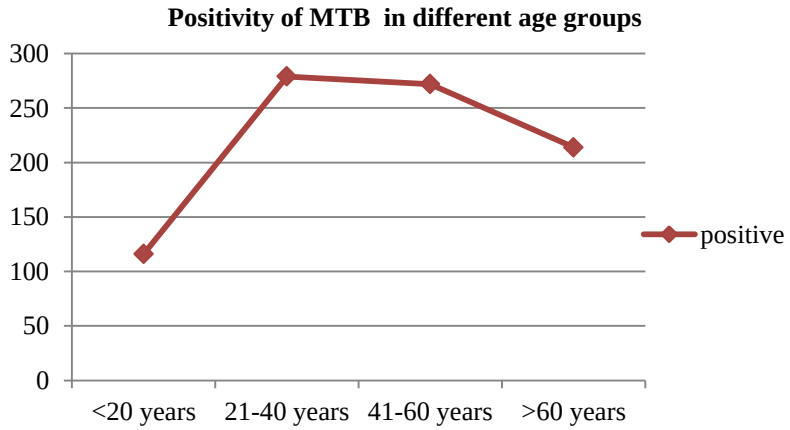


Fig. 1. Percentage of MTB in males and females of detected MTB specimen. *CSF = Cerebrospinal fluid, BAL= Bronchoalveolar lavage.

Tuberculosis in Different Age Groups

The MTB in different age group patients is shown in Graph 1. Effect of age on the spread of disease was evaluated. It was observed that the age group of 21 to 40 years represented the majority of the MTB positive samples 279 (31.67%) while a slightly lower positive samples 272 (30.87%) were observed in the age group of 41 to 60 years.



Graph 1. The percentage of positivity of MTB in different age groups.

Discussion

The lungs and respiratory system can be usually affected by MTB, and it can spread by the water droplet as aerosols and can localize to any other organs and tissues. So, site specific specimen is required to detect MTB, such as for pulmonary TB sputum or Bronchial wash or Bronchoalveolar lavage (BAL) is used, but for extra pulmonary TB, urine for kidney infection, CSF for nervous system, Synovial fluid for joint infection etc. are used for the detection of TB (Toyoda *et al.*, 1996) . But some patients having TB like symptoms such as fever, malaise, wight loss etc. but no specific manifestations for TB diseases. For these patients peripheral blood samples are used for the detection of MTB by GeneXpert PCR assay but each and every time *M. tuberculosis* remains undetected. These undetected samples of some patients may not be infected by the *M. tuberculosis,* and in some cases, there are few *M. tuberculosis* infection observed due to infection in the intestine or stomach (Hamer *et al*., 2008). In these cases, Endoscopy will be more helpful to find any lesion and subsequently a tissue biopsy can be used for the detection of MTB. The treatment of tuberculosis (TB), prevention and control can be achieved by rapid and accurate detection at the immediate onset of TB. In this current study, we had investigated the *M. tuberculosis* (MTB) from pulmonary, extra pulmonary and peripheral blood samples by GeneXpert PCR assay. We found MTB in both pulmonary and extra-pulmonary samples in various percentages except peripheral blood samples. A small difference was observed in male and female of MTB positive patients. Moreover about 62.54% MTB positive patients were observed in the age group between 21 and 60 years and might be the potential carrier and spreader of MTB. Early and accurate detection of MTB with proper sample at the onset of the disease might be helpful to cure and control the spread of infection further. It might be indicated that peripheral blood sample might not be a sample of good choice for MTB detection.

Conclusion

We can conclude that GeneXpert PCR assay can be reliably employed for quick and early detection of MTB in all types of fluid and tissue samples from patients with a clinical suspicion of tuberculosis (pulmonary or extra pulmonary) except in peripheral blood. Further investigation may be needed to establish any other customize protocol for effective use of peripheral blood as clinical specimen for MTB detection by GeneXpert PCR system or any other Real Time PCR system.

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