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Diagnostic performance of isothermal strand displacement amplification of *Mycobacterium tuberculosis* IS 6110 in tissue samples

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ARTICLE INFO

Article history:
Received 18 August 2012
Accepted 1 September 2012
Available online 5 October 2012

Keywords:
Strand displacement amplification
Tuberculosis
NAAT-TB
Histopathology
Microbiology

ABSTRACT

Background: Visualized histopathological findings in tissue samples are not specific for tuberculosis while mycobacterial cultures from such specimens have low yields and long turn around times. A rapid, sensitive method is therefore needed for detection of *Mycobacterium tuberculosis* in paucibacillary tissue samples.

Methodology: In this paper, a total of 158 tissue specimens, including 42 culture-positives, were tested for the presence of *Mycobacterium tuberculosis* by strand displacement amplification of DNA targeting the region of the insertion element IS 6110 and detected by a chemiluminescence based commercial platform (BDProbeTec™ ET System). The amplification results were correlated to histopathology, microscopy and microbiological culture.

Results: The strand displacement amplification based assay showed low overall sensitivity (31.5%) but high specificity (97.5%) which varied across various tissue types. Only 35.7% of culture-positive biopsies were positive by the molecular assay. Some discrepancy were attributed to suboptimal performance of the traditional methods.

Conclusions: The assay is useful to rule in the disease in common tissue specimens (lung, pleura and lymph node); but less so in other tissue types. The poor sensitivity in tissue specimens necessitates careful interpretation of data generated by the assay in conjunction with a clinical suspicion of tuberculosis for making decision regarding empirical treatment. The complexity of the disease pathology along with the low bacillary load and clumping tendency require selection of more sensitive methods or gene targets.

Introduction

Diagnosis of tuberculosis (TB) in tissues represents a diagnostic challenge since the disease tends to be paucibacillary with a subsequent low yield of acid-fast microscopy [1,2]. While bacteriological confirmation is helpful and essential for speciation and susceptibility testing, it is time-consuming owing to the long generation time of the organism, as well as the technical difficulties in growing mycobacteria from tissue samples. For these reasons, a rapid histopathological diagnosis of TB is valuable to initiate treatment promptly and apply appropriate infection control measures. In additions, rapid diagnosis of TB has a considerable cost impact on healthcare institutions [3].

Mycobacterium tuberculosis bacilli are visualized in tissue sections using specialized stains, including the traditional Ziehl-Neelsen stain. Diagnosis of TB from representative tissue biopsy samples is supported by the presence of caseating

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2212-5531/$ - see front matter
http://dx.doi.org/10.1016/j.ijmyco.2012.09.001

granuloma, the classic histopathological picture of TB. A toxic glycolipid component of the mycobacterial cell wall is thought to induce caseation [4]. While the presence of caseous necrosis is highly suggestive of TB, this finding can be apparently confused with invasive mycoses, such as cryptococcosis and histoplasmosis. Necrotizing non-granulomatous lesions can also be attributed to tissue TB [5], and thus can be confused with other necrotizing pathological lesions. Furthermore, the histopathological features of TB may show considerable variation and are largely dependent on the underlying immune response [6]. The anergic histopathological presentation of TB is being increasingly recognized with the advent of the era of immunosuppressed patients, making the distinction between TB and other chronic conditions particularly difficult on morphological grounds alone. This shows the critical need for rapid, accurate tissue diagnostics, such as the nucleic acid amplification tests for TB (NAATs-TB). The chief role of those assays is a rapid mean for presumptive diagnosis of TB, which justifies the initiation of therapy in cases where clinical judgment alone does not favor doing so. Genetic targets that have been used for detecting *M. tuberculosis* include IS 6110, 65KD heat shock protein, MPB 64, 38KD protein and ribosomal RNA [7]. Various molecular approaches have been successfully applied for detecting mycobacterial DNA by commercial platforms with the principle based on either conventional PCR techniques followed by sequencing of the amplified product, solid phase hybridization, or real-time amplification methods like the strand displacement amplification (SDA). The advantages of such systems are better quality control of reagents, user-friendly format and potential of automation [8]. However, these commercial systems have been inadequately validated in non-respiratory specimens, and the accuracy of NAATs-TB in this context remains unclear with previous reporting of inadequate diagnostic accuracy and, in particular, false positives in various clinical settings [9]. Of significance is the poor sensitivity in detecting mycobacterial DNA in clinical specimens compared with cultures [10].

The SDA, an isothermal amplification assay, is based on the ability of the Klenow fragment of *Escherichia coli* DNA polymerase to start at the site of a single stranded nick in double stranded DNA. This is followed by extending one strand from the 3^- end and displacement of the downstream strand of the DNA [11]. The replicated DNA and the displaced strands are then substrates for further annealing, nicking and strand displacement. This results in geometric amplification ($\sim 10^8$ folds amplification reaction in 2 h). Thus, the SDA process has a low detection limit of around 10–50 copies, so when the target is present in several copies in the genome of the organism (e.g. IS 6110 in *M. tuberculosis*), only a few number of the bacterium is required for detection [12]. The BDProbeTec system (Becton Dickinson), adopted in this study, provides a mycobacterial-specific platform for SDA coupled with a chemiluminescence detection system. This allows the whole sensitive assay to be performed in 4 h following the processing and decontamination of specimens if required, so the assay is a convenient alternative for real-time PCR [13]. Since its initial description and evolvement, the ProbeTec has been evaluated mainly in respiratory specimens, but also in non-respiratory fluid samples [14,15]. Limited data, however, exist regarding its performance in tissue biopsies. A study was conducted to evaluate the diagnostic performance of the BDProbeTec system in various tissue samples and its concordance with histopathological examination (HPE) and bacteriological culture.

Materials and methods

Study settings and specimens

This comparative study was conducted between and including January 2011 and April 2012 in the Diagnostic Laboratories of a tertiary care center in Saudi Arabia. One hundred and fifty-eight tissue samples were processed for both SDA and HPE. Tissue samples for SDA were sent in normal saline and for histopathology in 10% formalin. All the samples were kept at 4 °C before processing.

Bacteriological methods

All the tissue biopsies were dissected, manually macerated and homogenized using a sterile tissue grinder then subjected to decontamination by a standard *N*-acetyl-L-cysteine (NALC) digestion method. Briefly, specimens were treated with an equal volume of NALC–NaOH (final concentration 2%) for 15 min at room temperature, neutralized with sterile phosphate buffer (pH 6.8), and centrifuged at 3000*g* for 30 min. The sediment pellet was then re-suspended in 2 ml phosphate buffer and subjected to both culture and SDA analysis using the BDProbeTec ET system following manufacturer's instructions. Culture was performed using two Löwenstein-Jensen slopes (with and without pyruvate), and the MGIT (Becton Dickinson) liquid culture system according to the manufacturer's instructions. All specimens were screened microscopically after concentration using the Auramine stain with positive results confirmed using Ziehl-Neelsen staining.

Molecular methods

Following the manufacturer's instructions, 500 μl post-decontamination specimens were washed with 1 ml wash buffer 1, and then centrifuged at 12,200*g* for 3 min. The supernatant was discarded and mycobacteria were subjected to killing by heat (105 °C) for 30 min. DNA was released from the organisms by re-suspending the deposit in 100 μl lysis buffer 2, followed by sonication at 65 °C for 45 min. Samples were then neutralized by adding 600 μl neutralization buffer. A volume equivalent to 150 μl DNA extract was added to the priming well containing dehydrated primers and probes in the microtiter plate. The plate was incubated at room temperature for 20 min to allow complete rehydration of reagents. The priming mix was incubated at 72.5 °C for 10 min. Enzymes, dNTPs and buffer were activated in a separate amplification microtiter plate by heating to 54 °C for 10 min. Hundred microliter of the priming mix was added to each corresponding well and mixed to initiate amplification. Plates were then transferred to the BDProbeTec analyzer. Each assay run was done in duplicate and included positive and negative controls, along with the assay supplied internal control to avoid false-nega-