Abstract

Assessment of Detection Efficacy of *Mycobacterium tuberculosis* in sputum samples by Real Time PCR based method

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Abstract

A baseline study involving thirty (30) sputum samples from suspected pulmonary tuberculosis (TB) patients is collected and performed Acid Fast Bacilli (AFB) tests, culture till it shows growth (for six to eight weeks) and comparing these results with those obtained using Real Time PCR detection.

AFB tests were carried out on 30 samples at the Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal; Real time PCR (QPCR) methodology at Center for Molecular Dynamics Nepal (CMDN) affiliated Intrepid Nepal (IN), Kathmandu, Nepal and Culture at Kathmandu University, Dhusikhel, Kavre, Nepal. The microbiological assessments applied were as per WHO guidelines (Ziehl-Neelsen staining or AFB staining) and culture was used as a Gold standard for the sensitivity and specificity assessment. The QPCR assay used targeted IS6110, a 12.7 Kb fragment of M. tuberculosis not found in other Mycobacterium sub-species. Thirteen samples (43%) were found to be AFB positive and Seventeen (57%) samples were AFB negative. Fourteen of the samples (47%) were PCR positive and Sixteen (53%) were PCR Negative. Three of AFB negative samples were found to be PCR positive. Two AFB positive samples were found to be PCR negative. Thirteen samples (43%) were found to be Culture Positive and Seventeen samples (57%) were found to be Culture Negative. One Culture Negative samples was found to be PCR positive. Two Culture Negative samples were found to be AFB positive and two culture positive samples were found to be AFB Negative. The sensitivity with respect to gold standard (culture) for AFB was calculated to be 84.61% while for Q-PCR it was calculated to be 100%; specificity for AFB 88.24% while for Q-PCR 94.11%; Positive predictive value for AFB was found to be 84.61% while for Q-PCR, it was calculated to be 92.86%; Negative predictive value was found to be 88.24%, while for Q-PCR, it was calculated to be 100%. These statistics clearly show that Q-PCR is highly efficient for the diagnosis of TB compared to AFB.

The findings from this study demonstrates the need to deploy highly specific and sensitive genomic based method of detection of M. tuberculosis in conjunction with traditional AFB, X-ray, Tuberculin test, Fluorescein test, culture tests etc done for TB detection to come up with rapid, reliable and accurate detection of M. Tuberculosis in Nepal. Since QPCR will help this issue in the context of Nepal, the technology of