Multiplex Allele Specific-PCR Methods for Fast Detection of GyrA Gene Mutaions in Clinical Isolates of Mycobacterium Tuberculosis

Vahideh Vahidi[1]; Mohammad Reza Zolfaghari*[1]; Reza Moadab[2]; Azam Ahmadi[3]; Mohammad Arjomandzadegan[4]

1-Department of Microbiology, Qom Branch , Islamic Azad University,Qom, Iran

2- Center for Tuberculosis Research, Faculty of Tabriz University of Medical Sciences, Tabriz, Iran

3- Department of Molecular Cell Biology, Isfahan University, Isfahan, Iran

4-Department of Microbiology, Tuberculosis and Infectious Research Center, Arak University of Medical Sciences, Arak, Iran

mmatinam81@yahoo.com

Background & Objectives: Two molecular methods were designed and compared for rapid detection of resistance to ofloxacin in clinical isolates of *Mycobacterium tuberculosis*.

Methods: From 136 *M. tuberculosis* clinical isolates, 41 strains were used for comparison of detection of mutations associated with resistance in gyrA gene by Allele Specific-PCR (AS PCR), Multiplex Allele Specific-PCR (MAS PCR) and Semi-nested Allele Specific-PCR (SnAS PCR). Specific internal primers were designed for detection any changes in 90, 91 and 94 codons. Sequencing methods were accomplished for evaluation of the results as gold standard.

Results: AS-PCR and SnAS-PCR methods could detect mutations by formation or not formation of internal bounds. MAS-PCR by like mechanism has good performance. Totally, from 37 strains phenotypically resistant to ofloxacine 32 strains were mutant and 5 strains were non mutant that have sensitivity and specificity, 86/11% and 100% , respectively. Sequence results were concordant by results molecular methods.

Conclusion: Results of the study showed that MAS-PCR methods could be use as a routine test for fast detection of resistance to fluroquinolones in *M. tuberculosis*

Keywords: Multiplex Allele Specific; PCR (MAS PCR); *Mycobacterium tuberculosis*; Ofloxcacin