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Letter to Editor

Recurrence after treatment success in pulmonary multidrug-resistant tuberculosis: predication by continual PCR positivity

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Letter to Editor:

Over the last decade, drug–resistant tuberculosis (TB) becomes a growing threat to public health despite advances in treatment and diagnosis. TB strains resistant to the first line drugs isoniazid and rifampin, called multidrug-resistant (MDR-TB), now account for more than 5% of all TB cases globally [1]. Generally, the standard treatment for MDR-TB patients mainly comprised a combination of first and second line drugs [2]. The drugs consisted of amikacin, prothionamide, ofloxacin and cycloserine [2]. Ethambutol and pyrazinamide were added to treatment if mycobacterium was sensitive to them. The treatment period continued for 18 months after achieving the first negative sputum culture, or at least 24 months when no first line drug was used. Follow-up evaluations included a sputum smear and culture examination every month along with chest X-ray every three months [2]. Although till date, no randomized control trials were performed to evaluate the success of treatment of MDR-TB in Iran, but in account of susceptibility test and clinical – outcomes for individual patients, the cure rate stated to be from 60 to 70% [3, 4]. Recently, among sixty five MDR-TB patients who were completed treatment, 23 experienced an episode of recurrent tuberculosis (23/65; 35%). Notably, all of these patients had continual polymerase chain reaction (PCR) positivity reports (based on IS6110 assay), while their cultures remained negative well beyond 12 months of therapy. In contrary, recurrence did not appear in patients who had negative reports for both culture and PCR-assay (42/65; 64%). Basically, molecular assay based on *Mycobacterium tuberculosis* DNA are not useful in monitoring response to therapy since the DNA found after treatment period may come from persistent free DNA or free dead bacilli, as postulated in a chronic tuberculosis model [5]. Although this is possible, it is also possible that this DNA represents metabolically dormant forms of bacilli. In this regards, more recently Hernandez-Pando *et al*., detected DNA of *M. tuberculosis* in cadaveric lungs. He further proposed that this bacterium can persist intracellularly without histological evidence of tuberculosis lesions [6]. To find-out whether the reported discrepancies (culture negative and PCR positive) among recurrence cases in our study was due to laboratory error , presence of dead-bacilli , or persisters cell, the sequential sputum samples were analyzed using Atomic Force Microscopy (AFM) [7]. Briefly, the sputum samples (n=23) were divided into two equal parts; one used for smear, culture and PCR - analysis and the remaining were passed through Millipore filter (0.2 µm) and used for AFM investigation. We detected mycobacterial persister like cells in 91% (21/23; P>0.05) of the studied sputum samples (**Figure 1**). To specifically increase their

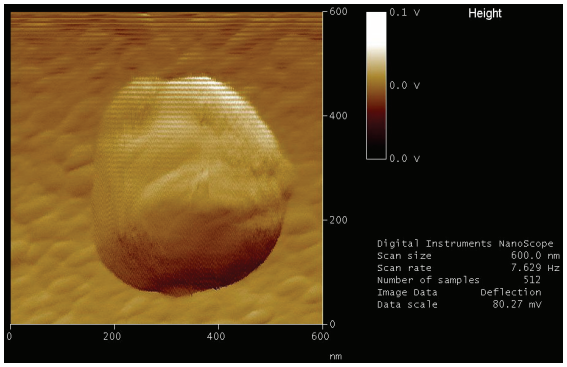

Figure 1: AFM shows that continual PCR positivity in pulmonary multidrug -resistant tuberculosis is because of phenotypical modified persister's cells.

attachment to the silicon plates, the surface was charged with polyclonal Rabbit anti-mycobacterium (Dako; B0124). Persisters–like TB bacilli (150 to 300μm) were small round or oval in shape and their numbers were variable in different specimens ($\approx 1X10^{2-4}$). In overall, our findings suggest that although majority of MDR-TB bacilli are killed by treatment, nevertheless a small number may go under phenotypic changes to tolerate the cidal effect of the anti-mycobacterials. In this case, the likely reason behind the continual PCR positivity is the presence of phenotypical modified "persisters" rather than free DNA shedding. Based on this preliminary result continual PCR positivity, even when the culture is negative should be seriously taken in consideration as it may reveal treatment failure in MDR-TB patients in otherwise clinically non –suspicious of treatment failure.

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References

[1] World Health Organization. Multidrug and extensively drug –resistant TB (M/XDR-TB):2010 Global Report on Surveillance and Response. Geneva, Switzerland.

[2] Mirsaeidi SM, Tabarsi P, Khoshnood K, Pooramiri MV, Rowhani-Rahbar A, Mansoori SD, Masjedi H, Zahirifard S, Mohammadi F, Farnia P, Masjedi MR, Velayati AA. Treatment of multiple drug –resistant tuberculosis (MDR-TB) in Iran. Int J Infect Dis 2005; 9:317-322.

[3] Tabarsi P, Chitsaz E, Tabatabaei V, Baghaei P, Shamaei M, Farnia P, Marjani M, Kazempour M, Mansouri D, Masjedi MR, Velayati AA. Revised Category II regimen as an alternative strategy for retreatment of Category I regimen failure and irregular treatment cases. Am J Ther 2011; 18:343-349.

[4] Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, Ziazarifi AH, Hoffner SE. Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in iran. Chest 2009; 136:420-425.

[5] Wit D de, Wootton M, Dhillon J, Mitchison DA. The bacterial DNA content of mouse organs in the cornell model of dormant tuberculosis. Tubercle Lung Dis 1995; 76:555-562.

[6] Hernández-Pando R, Jeyanathan M, Mengistu G, Aguilar D, Orozco H, Harboe M, Rook GA, Bjune G. Persistence of DNA from Mycobacterium tuberculosis in superficially normal lung tissue during latent infection.Lancet 2000;356:2133-2138.

[7] Velayati AA, Farnia P, Merza MA, Zhavnerko GK, Tabarsi P, Titov LP, Ghanavei J, Farnia P, Setare M, Poleschuyk NN, Owlia P, Sheikolslami M, Ranjbar R, Masjedi MR. New insight into extremely drug-resistant tuberculosis: using atomic force microscopy. Eur Respir J 2010; 36:1490-1493.