ORIGINAL ARTICLE

Genotyping and molecular detection of multidrug-resistant *Mycobacterium tuberculosis* among tuberculosis lymphadenitis cases in Addis Ababa, Ethiopia

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Abstract

Multidrug-resistant tuberculosis (MDR-TB) has emerged as a major public health problem. Drug-resistance surveillance data show that 3.9% of new and 21% of previously treated TB cases were estimated to have had rifampicin/ multidrug-resistant tuberculosis (MDR/RR-TB) in 2015. This implies that the MDR-TB is increasing alarmingly. Hence, a better understanding of drug resistance mechanisms and genotypes associated with multidrug resistance in *M. tuberculosis* is crucial for improving diagnostic and therapeutic methods to treat individuals with MDR-TB. The aim of this study was to analyze molecular drug resistance mutations of MDR-TB isolates from the cases of TB-lymphadenitis in relation to its genetic lineages.

A cross-sectional study was conducted on culture positive cases from July to October, 2014 in Addis Ababa, Ethiopia. Sixty isolates were included to analyze drug resistance mutated gene responsible for MDR-TB in relation to its molecular genotyping. Mycobacterial culture, GenoTypeMTBDR plus and Spoligotyping were used to undertake the study.

Of 60 TBLN isolates, 8.3% were identified MDR-TB cases and one isolate was isoniazid mono-resistant. Eleven isolates in T3-ETH genetic sub lineage were sensitive to both RMP and INH, while only 2 isolates were MDR-TB. Most of the RMP- resistant isolates showed mutation in codon S531L and all isolates mutated in the *katG* gene conferring INH resistant strains had mutations in codon of S315T1.

Screening for the *rpoB* and *katG* gene mutation of tuberculosis lymphadenitis is useful in Ethiopia for an early detection and treatment of MDR-TB. Besides, there is a drug resistance variation among different lineages of Tuberculosis lymphadenitis which has important consequences for the development of efficient control strategies.

Key words: Addis Ababa, genotyping, isoniazid, multidrug-resistant tuberculosis, rifampicin, tuberculosis lymphadenitis
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Introduction

In 2015, there were an estimated 480 000 new cases of multidrug-resistant tuberculosis (MDR-TB) and 250 000 deaths because of rifampicin/ multidrug-resistant tuberculosis (MDR/RR-TB). Drug-resistance surveillance data show that 3.9% of new and 21% of previously treated TB cases were estimated to have had MDR/RR-TB in 2015 [1]. MDR-TB is defined as a type of TB that shows resistance at least to rifampicin and isoniazid and it threatens TB control programmes in many parts of the world [2]. It has been reported that in Ethiopia the proportion of new cases with MDR-TB is 2.7% higher among people previously treated for TB, at 14% in 2015 [3].

A better understanding of drug-resistance mechanisms in *Mycobacterium tuberculosis* infections is crucial for the

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development of rapid methods for drug-resistance detection and new anti-TB drugs, which are used to treat patients with MDR-TB. Early molecular studies identified *katG* (encoding catalase peroxidase) and *rpoB* (encoding the β-subunit of RNA polymerase) genes as major targets conferring resistance of *M. tuberculosis* to isoniazid and rifampicin, respectively [4,5]. The increased incidence of MDR-TB cases, particularly in high-burden countries like Ethiopia, raised the need for implementation of rapid molecular methods as an alternative to culture-based drug susceptibility testing. Recently, WHO recommended the use of molecular techniques such as line probe assay and GeneXpert assay for rapid screening of MDR-TB to evaluate the presence of genomic mutations conferring resistance in MDR-TB [6,7].

Some published data on MDR-TB are available in Ethiopia but limited studies on molecular detection of MDR-TB lymphadenitis (TBLN) [8,9] have been conducted and no data are found regarding genotyping characterization of specific lineage in relation to MDR-TBLN because it was forgotten that extra pulmonary TB, particularly TBLN, can also form pulmonary TB. The present study, therefore, tried to assess MDR-TB in suspected TBLN by using line probe assay, which is similar to the GeneXpert assay for detection of the mutation in specific genes responsible for the drug-resistance and also to identify the relationship of specific TB lineages to MDR-TB among TBLN isolates.

Materials and methods

Patients' recruitment and study settings

A cross-sectional study was conducted on culture-positive cases from July to October 2014 in Addis Ababa, Ethiopia at Tikur Anbessa Specialized Hospital and Alem Tena Higher Clinic, these sites were purposely selected because more patients with TBLN were referred to them. A total of 65 isolates were included to analyse the gene responsible for MDR-TB in relation to its molecular genotyping at the Ethiopia Public Health Institute. This institute is well-known in Ethiopia for conducting research and the molecular TB research centre is part of it. To this effect, demographic data were collected from all patients using a pre-structured questionnaire by trained clinical nurses. Patients on anti-TB treatment and <18 years old at the time of sample collection were excluded.

Sample collection and processing

Fine needle aspiration (FNA) samples were collected by a pathologist from the affected nodes. Briefly, the swollen area was cleaned with 70% alcohol and then a 21-gauge needle was inserted into the mass. After removing the needle, drops of aspirate were placed on a clean slide for FNA cytology, thereafter, leftover FNA samples were added aseptically into sterile universal tubes in PBS, pH 7.2, at 4°C.

Mycobacteriological culture

Samples suspected of TBLN were processed and cultured at the Aklilu Lemma Institute of Pathobiology TB laboratory. Briefly, FNA specimens were homogenized and mixed with 0.85% normal saline and decontaminated by shaking in an equal volume of 4% NaOH for 15 min at room temperature. Then, the specimens were subjected to centrifugation at 3000 rpm for 15 minutes. After homogenization, 100 µl of the sediment was inoculated into the conventional Lowenstein–Jensen egg slant medium, containing 0.6% sodium pyruvate. The cultures were incubated at 37°C in a slanted position for 1 week and in the upright position for 4–8 weeks. the same media. Culture of the specimen was incubated at 37°C for 3–8 weeks. The media were checked for evidence of bacterial growth daily for the first week and weekly for the rest of the time until 8 weeks. Microscopic examination of the colonies was performed using Ziehl–Neelsen stain to select Acid Fast Bacilli positive isolates. Heat-killed cells were prepared by mixing two loops of colonies in 200 µl distilled water and thereafter heating at 80°C for 1 h. The heat-killed cells were used for molecular characterization.

To check for the quality of the Lowenstein–Jensen medium, a fast-growing mycobacterium complex strain was run in parallel with each batch of inoculated media. In addition to this, uninoculated Lowenstein–Jensen tubes were incubated at the same time to control for contamination.

Spoligotyping

Spoligotyping was carried out using a commercially available kit from Ocimum Biosolutions (UK) described earlier [10]. To put it briefly the direct-repeat (DR) region was amplified with primers DRa and DRb. Then, the amplified DNA was denatured and the single-stranded DNA was hybridized with 43 spacer oligonucleotides covalently bound to a membrane. For running spoligotyping the already published procedure was followed [11]. DNA from *Mycobacterium .bovis*, bacillus Calmette–Guérin and *M. tuberculosis* H37Rv were used as positive controls, whereas Qiagen water (Qiagen, Hilden, Germany) was used as a negative control. The presence of spacers was visualized on film as black squares whereas the absence of spacers was indicated by white squares following incubation with streptavidin-peroxidase and they were detected by the enhanced chemoluminescence system detection liquid (Amersham, Little Chalfont, UK). Spoligotype patterns of each strain were prepared in binary and octal format and entered into the recently released international spoligotyping database SITVITWEB [12] for detecting their spoligotype International Type (SIT) numbers. Strains for which SIT numbers could not be recovered from the database were considered orphans. Lineage and sublinage designation were according to SPOTCLUST at http://tbinsight.cs.rpi.edu/run_spotclust.html. All the steps of sample processing and PCR were carried out in a separate PCR