Viable Mycobacterium tuberculosis in sputum after pulmonary tuberculosis cure

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Research article

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Abstract

Background Pulmonary tuberculosis (TB) with detectable Mycobacterium tuberculosis in the sputum is a major source of transmission. In resource limited TB endemic settings, cure is declared by sputum smear examination for acid fast bacilli without performing culture. This may lead to erroneous treatment outcomes as viable bacteria may be missed by low sensitivity of direct smear method of acid fast staining. The aim of this study was to investigate if sterilizing cure is achieved among the new pulmonary TB cases declared cured by sputum smear conversion and the impact of addition of ethambutol in the continuation phase in achieving sterilizing cure. Methods New sputum smear positive pulmonary TB patients registered at a tertiary care hospital in Pakistan were followed under standard Directly Observed Treatment Short Course strategy for six months. Half of these patients received ethambutol in addition to isoniazid and rifampicin in continuation phase. Sputum specimens were examined on microscopy at 2 months and end of treatment. Sputa of patients with negative direct smear examination at the end of treatment were cultured on solid medium. Results Total of 533 newly diagnosed sputum smear positive pulmonary TB patients were registered from November 2013 to March 2014. Among these 504 converted sputum negative at 2 months and 348 converted at the end of six months of treatment and declared cured. Sputa of 204/348 patients were cultured, and 12/204 (6%) were culture positive. Culture positivity at 6 month was not associated with bacterial load, smoking, diabetes, presence of cavities, history of contact with TB, age, gender, socioeconomic status, or addition of ethambutol in the continuation phase of treatment. Conclusion Six month treatment does not provide sterilizing cure in all pulmonary TB leading to risk for relapse. Direct smear examination is not enough to declare cure in TB patients. Addition of ethambutol in the continuation phase did not result in better sterilizing cure. These findings emphasize the importance of performing culture and follow-up of patients to monitor relapse in routine TB care. More studies are needed to find the optimal duration of treatment for individual or carefully selected groups of patients.

Background

Tuberculosis (TB) is a major global health concern. As per World Health Organization (WHO) TB report in 2018, annual global incidence has not decreased below 10.0 million [1]. Pulmonary TB with detectable *Mycobacterium tuberculosis* (MTB) in the sputum is a major source of transmission, therefore focus of global TB Control strategies. Sputum smear microscopy for acid-fast bacilli (AFB) is a widely available, simple, and inexpensive tool for pulmonary TB diagnosis and treatment monitoring [2]. The standard treatment for TB comprises an intensive phase with isoniazid (INH), rifampicin, pyrazinamide, and ethambutol for 2 months, followed by a continuation phase that comprises the concomitant use of INH and rifampicin for another 4 months [3, 4]. This standard treatment period of 6 months was determined by acceptable rates of treatment failure and disease recurrence after discontinuation of chemotherapy, and is considered effective for drug-susceptible TB [5]. Response to TB treatment is monitored by follow-up sputum smear microscopy at 2 months and five months [4, 6]. Diminishing numbers of AFB to smear-negative status during treatment are considered an indication of treatment success . A negative sputum

smear during the last months of treatment is considered as cure. In resource limited settings, Mycobacterial culture is not routinely performed on sputum smear-positive cases for monitoring treatment response [4]. There is little information if bacterial sterilization is achieved after 6 months of treatment in all cases [7] and if sputum smear conversion is a satisfactory method for measuring sterilizing cure.

Minimum two effective drugs are necessary in the continuation phase of treatment to achieve successful treatment outcome and to prevent emergence of multidrug resistance strains. Drug resistance survey in Pakistan in 2016 has shown 7% INH resistance among rifampicin sensitive cases [8]. This implies that about 7% of the pulmonary TB cases would be receiving only one effective drug in the continuation phase. This could contribute towards failure to achieve the sterilization and persistence of a small number of bacilli even after treatment and emergence of resistant strains. According to WHO, ethambutol can be added in continuation phase of patients with known or suspected high levels of INH resistance, but more evidence is needed to support this recommendation [4]. The aim of this study was to investigate if sterilizing cure is achieved among the new pulmonary TB cases declared cured by sputum smear conversion and the impact of addition of ethambutol in the continuation phase in achieving sterilizing cure.

Methods

Study setting and design

Study was conducted at Gulab Devi hospital (GDH), from November 2013 to March 2014. GDH is a private not-for-profit large tertiary care hospital located in Lahore, the capital city of the country's largest province. GDH is known for specialized TB care and presumptive and diagnosed TB patients are referred from various districts to GDH for consultation and /or treatment. Many patients after the diagnosis are referred back to TB clinics close to their residence for treatment.

This study was nested in another larger project. New sputum smear positive pulmonary TB patients without a history of previous TB treatment were included if they had successful sputum smear conversion at the end of treatment and if the culture was performed. All these patients were given standard anti-TB treatment as per WHO guidelines [4]. Rifampicin, INH, ethambutol and pyrazinamide were given for initial two months (Intensive phase). After two month patients were split in two groups, one group was given rifampicin and INH for four months (continuation phase), while for other, ethambutol was added in continuation phase in addition to the INH and rifampicin. Follow up smears were done at second, fifth and sixth months of treatment. If sputum smear was positive at second month, examination was repeated at third month. Patients having negative sputum smear at the end of treatment were declared cured. These patients were enrolled in study if their sputa were sent for culture.

If the sputum smear was found to be positive at fifth or sixth month of treatment, case was declared as treatment failure, excluded from the study and referred to laboratory where *Xpert MTB/RIF* assay was performed. In cases where MTB deoxyribonucleic acid was detected and rifampicin resistance was not

shown, category 2 treatment was started according to the WHO guidelines [4]. In cases where rifampicin resistance was detected, patient was referred to the Programmatic Management of Drug-Resistant Tuberculosis site at GDH for further treatment and management.

Clinical and Laboratory investigations

Detailed clinical history, including history of smoking and diabetes and physical examination was performed. Baseline blood tests, human immunodeficiency virus (HIV) test and chest X-ray was done for patients during their first visit. Two (spot and early morning) sputum specimens were examined for the presence of AFB by Ziehl-Neelsen staining method and direct microscopic examination [9]. All investigations were done at GDH laboratory which is a quality assured laboratory participating in external quality assurance system. Two sputum samples were examined at the time of diagnosis, while single sputum was examined at each follow-up visit and end of treatment. The definition of a new sputum smear positive pulmonary TB case was based on the presence of at least one acid fast bacillus. The microscopy results were graded as per the guidelines of the International Union Against Tuberculosis and Lung Disease [10]. Sputa from the smear negative cases at the end of treatment were processed for cultures on Lowenstein-Jensen (LJ) medium. Sputa were decontaminated and concentrated and deposit was inoculated on two slopes of LJ medium [9]. Reading of culture slopes was done weekly. LJ culture tubes were kept for a maximum of 8 weeks at 37^0C before declaring them negative. Positive cultures were reported as soon as growth was detected. Identification was based on phenotypic appearance of colonies and acid fastness on Ziehl-Neelsen stained smears. Confirmation of *M. tuberculosis* complex was done by inhibition of growth in medium containing 0.5 mg/ml of para- nitrobenzoic acid [11]. In case of contamination in inoculated tubes, or growth of AFB mixed with contaminants, specimen /culture was reprocessed using N-acetyl-L-cysteine-sodium hydroxide method at final concentration of 2% sodium hydroxide [9].

Statistical Analysis

The data was entered into SPSS version 20 and cleaned for further analysis. Binary logistic regression analysis were carried out to identify factors associated with sterilizing cure. P value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 5746 patients were identified as presumptive TB cases from November 2013 to March 2014 (Figure 1). Among these 1738 (30%) were smear positive for AFB, and 1595 (92%) were new TB patients who were never treated for TB before, or had taken anti-TB drugs for less than 1 month. Among these 533 (34%) were registered at GDH. After 6 months of treatment, 348 (65%) patients were declared cured by sputum smear microscopy. Culture was performed on 204 of these patients. Demographic and clinical data of these 204 patients with available culture results is shown in Table 1. Relatively few (6.8%)

patients were smokers with predominance of male smokers. The prevalence of diabetes mellitus was low (5.4%), and none of these patients were positive for HIV. The majority of chest radiographs showed non-cavitary pulmonary infiltrates (93.1%), and cavities were seen in only 6.9% of cases. More than half of the patients belonged to low-income groups. History of TB contact from a household member was reported by 39% of the patients.

Treatment outcomes

After 2 months of treatment, sputa from 201/204 (98.5%) patients converted negative. At the end of 6 months of treatment, sputa from all patients converted negative. However 12 of these sputum smear negative cases had viable bacteria recovered on culture. Culture positivity at 6 month was not associated with bacterial load, smoking, diabetes, presence of cavities, history of contact with TB case, age, gender or socioeconomic status (Table 2).

Treatment outcomes with additional drug in the continuation phase

Patients who received additional ethambutol in the continuation phase had similar bacillary load and radiographic findings at the start of treatment as those who received the standard two drugs. The addition of ethambutol did not seem to have impact on sputum sterilization at the end of treatment.

Discussion

Standard 6 months of rifampicin containing treatment is considered curative for new drug sensitive pulmonary TB, and the sputum smear conversion is considered as a reliable marker for successful treatment. In this study, we show that sterilizing cure was not achieved in all cured patients after standard treatment, and viable cultivable bacilli were detected in 6% of patients despite successful sputum smear conversion. Earlier studies have shown that MTB may persist in lung tissue for months to years even after bacterial sterilization is achieved at the end of treatment [12-15]. A study has shown the presence of MTB mRNA in the context of non-resolving and intensifying lesions on positron emission tomography-computed tomography (PET–CT) images after treatment completion and bacterial sterilization suggesting that even apparently sterilizing curative treatment for TB may not eradicate all of the MTB bacteria in most patients [7]. The persisting bacilli could lead to relapse as shown in a study from Uganda where 10% of successfully treated patients with standard 6 months regimen patients got recurrence of TB within one year, and 81% of these recurrent TB cases were due to relapse [16]. The relapse rates ranges from 2.6% to 9.7% after successful standard treatment and sterilization cure [13, 16-18]. Based on these findings it can be speculated that relapse rate after non-sterilizing cure might be even higher. A study from Japan reported complete resolution of all active pulmonary TB lesions on PET-CT scan after 12 months of treatment, and no relapse at 1 year of follow-up [19]. These findings raise questions whether the presence of viable bacilli after treatment and relapse is related to the duration of treatment. The higher relapse rates in patient groups with impaired immunity support the concept that a competent immune response has an important complementary role in the ultimate control of residual bacteria after completion of antibiotic treatment [18, 20, 21].

Previous studies have shown that presence of lung cavities on chest X-ray at baseline is inversely proportional to sterilization at the end of treatment [22]. In our study no association was seen with cavities at start of treatment and sputum sterilization at 6 month of treatment. Culture positivity of sputum at 2 month has been shown to be associated with culture positivity at 6 month implying high bacterial load at the start of treatment could lead to unfavorable outcome [23]. We did not apply culture at 2 months but smear positivity at 2 months was not associated with culture positivity at 6 month, and neither was higher bacillary load at the start of treatment associated with sputum culture positivity at the end of treatment. However lack of these associations could be due to small number of patients with unfavorable outcome in our study.

In this study 5/12 culture positive cases had recent history of TB in family, implying the possibility of reinfection from the home environment rather than treatment failure as the source of culture positivity. Different studies in past have shown that exogenous reinfection could be a major cause of post primary TB after achieving cure especially in TB endemic areas [24]. People who had TB once are at an increased risk of developing TB when re-infected. Reinfection rate after successful treatment could be as high as four times that of new TB [25]. These findings emphasize the importance of achieving sterilizing cures and preventing transmission.

In our cohort, the proportion (5%) of diabetics among the TB patients was much lower than the prevalence of diabetes mellitus in general population in Pakistan, which is shown to be 26.3% [26]. This, and lack of association of diabetes with culture positivity at the end of treatment does not confirm the earlier studies indicating diabetes as a risk factor for active pulmonary TB and unfavorable treatment outcomes [20, 27, 28]. The prevalence of diabetes in our study could have been been underestimated as prevlance was based on patient history and serum glucose levels were not measured, leaving the possibility that some patients might had undiagnosed diabetes. A cross sectional study from Lahore, Pakistan found 14.8 % diabetics among TB patients by measuring serum glucose and HbA_1C levels [29]. This prevalence is higher than our cohort but still lower than the prevalence in general population. A Malaysian study reviewed 1267 active TB patients at a tertiary hospital, and did not find diabetes as a risk factor for treatment failure [30]. Thus diabetes may not be a substantial risk factor for all pulmonary TB and unfavorable treatment response.

Inadequate chemotherapy causing exposure of bacilli to a single effective drug can lead to selection of resistant subpopulations, and unfavorable treatment outcomes [31-33]. The national prevalence of 7% INH resistance among rifampicin sensitive cases in Pakistan implies that a substantial number of TB patients receive only one effective drug in the continuation phase [8]. However in our study, addition of ethambutol in the continuation phase had no effect on sterilization of sputum. Ethambutol is a bacteriostatic drug while INH is bactericidal. Despite WHO recommendation, the evidence to quantify the ability of ethambutol to achieve better outcomes when used in combination to “protect rifampicin” in patients with pretreatment INH resistance, is insufficient, and further research is needed.

There are some shortcomings of the study. It is a relatively small study, with a small number of unfavorable outcomes. Culture was not performed on many cases due to logistic reasons, and Chest X-ray results could not be retrieved for several patients. This could have added bias in the study. Patients were not followed- up after treatment to determine the recurrence of TB. Despite these shortcomings, the study gives useful information about the treatment outcomes in the routine TB control program settings, and could contribute towards evidence for improving the routine TB care.

Conclusions

This study shows that six month treatment does not provide sterilizing cure in all pulmonary TB patients leading to risk for relapse. Direct smear examination is not enough to declare cure in TB patients. Addition of ethambutol in continuation phase did not result in better sterilizing cure. These findings emphasize the importance of performing culture in routine TB care to define a case as cured. More studies are needed to find the optimal duration of treatment, and adjusting TB treatment for individual or carefully selected groups of patients, and the need for the development of improved pulmonary TB treatment strategies by better sterilizing drug or adjunct host-directed therapies.

List Of Abbreviations

AFB: Acid fast bacilli

CT: Computed tomography

GDH: Gulab Devi hospital

HIV: human immunodeficiency virus

INH: Isoniazid

MTB: *Mycobacterium tuberculosis*

PET: Positron emission tomography

TB: Tuberculosis

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the local ethical Committee of the Gulab Devi hospital. (project number 110/13 GDH). All study participants had given consent. Only verbal consent was obtained from the patients as the intervention was evaluated as a routine within the scope of the standard treatment

guidelines of World Health Organization. The ethics Committee had approved the verbal consent form. All participants were above16 years of age.

Consent to publish

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors` contributions

Concept and study design: TM, MJ, AA. Acquisition of data: MJ, AA, MAR. Overseeing data collection: MJ, MAR, Analysis and interpretation of data: TM, AA. Drafting and revising the manuscript: AA, TM. All authors read and approved the final manuscript

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Figures

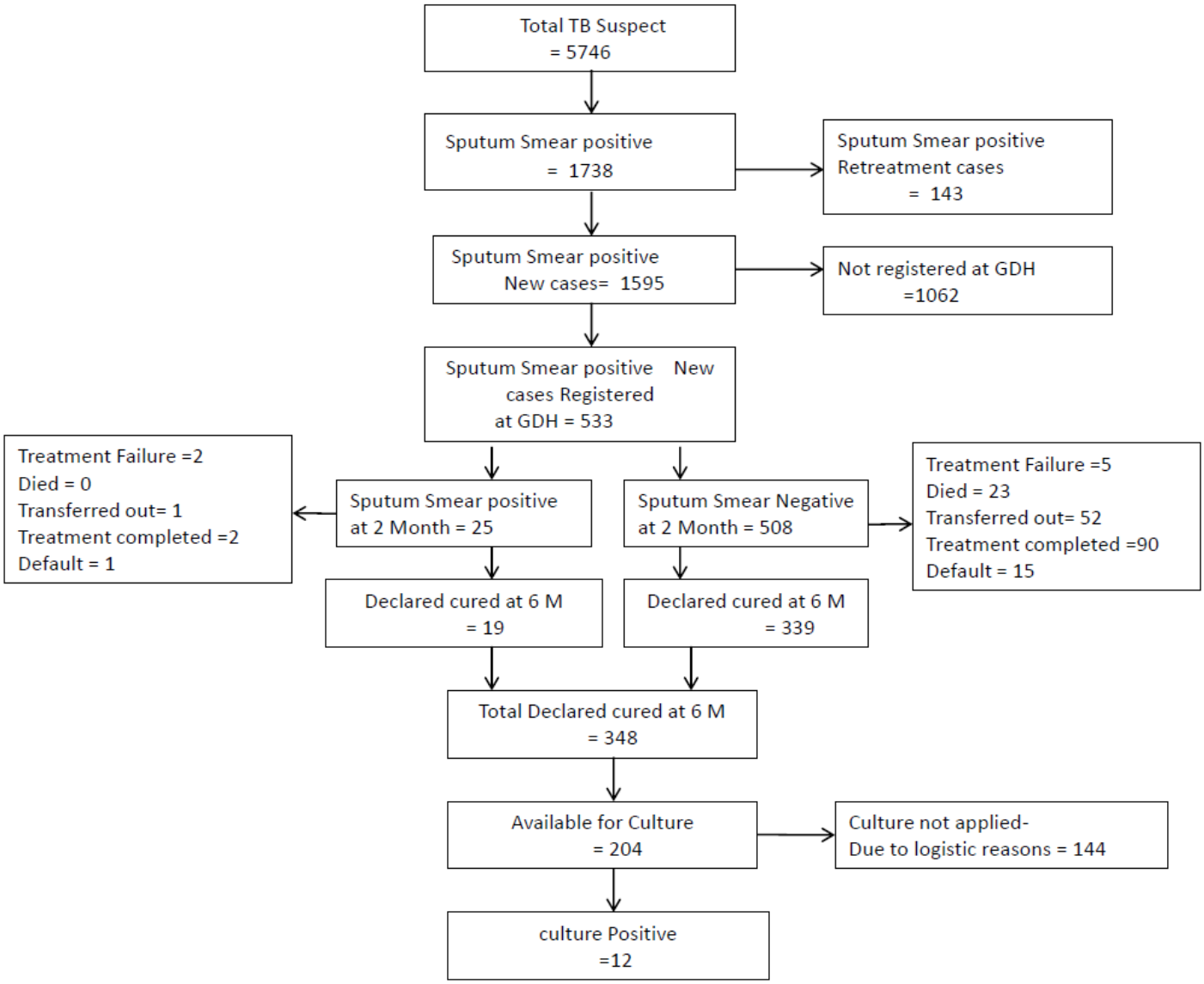

Figure 1

Flow chart of study design. GDH= Gulab Devi hospital, TB= Tuberculosis

Supplementary Files

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