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Implementation of LED Fluorescence Microscopy for Diagnosis of Pulmonary and HIV-Associated Tuberculosis in a Hospital Setting in Indonesia

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Abstract

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Background

Fluorescence microscopy (FM) has not been implemented widely in TB endemic settings and little evaluation has been done in HIV-infected patients. We evaluated diagnostic performance, time and costs of FM with light-emitting diodes technology (LED-FM), compared with conventional (Zieh-Neelsen) microscopy in a hospital in Indonesia which acts as referral centre for HIV-infected patients.

Method

We included pulmonary tuberculosis suspects from the outpatient and HIV clinic. Direct and concentrated sputum smears were examined using LED-FM and ZN microscopy by two technicians who were blinded for the HIV-status and the result of the comparative test. Mean reading time per slide was recorded and cost of each slide was calculated. Mycobacteria culture served as the reference standard.

Results

Among 404 tuberculosis suspects from the outpatient clinic and 256 from the HIV clinic, mycobacteria culture was positive in 12.6% and 27%, respectively. The optimal sensitivity of LED-FM was achieved by using a threshold of ≥2 AFB/length. LED-FM had a higher sensitivity (75.5% vs. 54.9%, P<0.01) but lower specificity (90.0% vs 96.6%, P<0.01) compared to ZN microscopy. HIV was associated with a lower sensitivity but similar specificity. The average reading time using LED-FM was significantly shorter (2.23±0.78 vs 5.82±1.60 minutes, P<0.01), while costs per slide were similar.

Conclusion

High sensitivity of LED-FM combined with shorter reading time of sputum smear slides make this method a potential alternative to ZN microscopy. Additional data on specificity are needed for effective implementation of this technique in high burden TB laboratories.

Introduction

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Microscopic observation of *Mycobacterium tuberculosis* in sputum smears still remains the mainstay of tuberculosis (TB) diagnosis in developing countries despite its poor sensitivity [1] especially among people infected with HIV because of their lower bacterial burden [2], [3]. Fluorescence microscopy (FM) of auramine-stained smears has been studied as an alternative to conventional light microscopy with Ziehl Neelsen staining (ZN). In a systematic review published in 2006, FM showed a similar specificity and on average 10% higher sensitivity than ZN staining [4]. FM however is not widely implemented in many TB-endemic settings, one reason being the high costs of the microscope. This has changed with the advent of cheaper fluorescent microscopes with light-emitting diodes (LED) [5]. Studies evaluating the performance of LED-FM have shown that in addition to the higher sensitivity, it had qualitative, operational and cost advantages over both conventional FM and ZN. On the basis of these findings, the World Health Organization recommended in 2011 to replace conventional FM by LED-FM and phase in LED-FM as an alternative to ZN microscopy [6].

In spite of this recommendation, policy makers and laboratory staff in many settings seem reluctant to introduce LED-FM for TB diagnosis. This is also true for Indonesia which has the fifth highest TB case-load worldwide. Issues including quality control, acceptibility, and ease may hamper its introduction. In addition, accuracy data for LED-FM in HIV-infected patients are scarce, with only one published study to our knowledge [7]. Also, WHO advocates a low threshold for positivity of FM (≥1 acid-fast bacillus/smear) [8], but this low threshold may contribute to the lower specificity of LED-FM compared to conventional microscopy reported by several studies [9], [10], [11]. Finally, only limited studies have evaluated the possible effect of sputum processing on the preformance of LED-FM [7], [12]. In conclusion, additional data are needed to define the optimal technical conditions of LED-FM, and its performance under field conditions and among HIV-infected patients [13], [14], [15].

We therefore defined the optimal threshold for positivity of LED-FM, examined the role of sputum concentration, and compared the performance of LED-FM and conventional light microscopy in patients with and without HIV infection, in terms of diagnostic accuracy, time and running costs under field conditions in Indonesia. Mycobacterial culture on Ogawa solid medium was applied as the reference standard.

Methods

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Ethics Statement

We used anonymized sputum samples collected as part of the study "The clinical features of pulmonary tuberculosis compared with pleural tuberculosis patients and the comparison of Auramin-stained method and Ziehl-Neelsen microscopy in both groups of patients" approved by the Ethical Committee of Hasan Sadikin Hospital/Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia (no. 91/FKUP-RSHS/KEPK/Kep/EC/2008). In addition, sputum samples were collected as part of laboratory assessment under an ongoing cohort study among HIV-infected patients in Hasan Sadikin hospital. Written informed consent is obtained from all patients in this cohort and this study has been approved by the Ethical Committee of Hasan Sadikin Hospital/Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia (no.114/FKUP-RSHS/KEPK/Kep/EC/2007).

Setting, Patients and Study Design

This study was conducted at Dr. Hasan Sadikin Hospital, the referral hospital for West Java Province, Indonesia. We included two groups of patients with suspected pulmonary TB, defined by the presence of cough ≥2 week duration with or without chest X-ray (CXR) abnormalities. The first group ('group 1') consisted of 527 outpatients, not (yet) taking TB treatment and with a very low risk of being HIV-infected. HIV testing is not routinely done for all pulmonary TB suspects in Indonesia. So far, Indonesia