SHORT COMMUNICATION

LED fluorescence microscopy increases the detection of smear-positive pulmonary tuberculosis in medical colleges of India

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In July 2012, light-emitting diode fluorescence microscopy (LED-FM) replaced conventional light microscopy using Ziehl-Neelsen stain in the detection of sputum-positive pulmonary tuberculosis in 190 microscopy centres of medical colleges operating under India's Revised National Tuberculosis Control Programme. We compared the performance of LED-FM (July–December 2012) to that of conventional microscopy (July–December 2011) across 190 sites. Of 222 658 patients examined using conventional microscopy, 28 042 (12.6%) were smear-positive, while of 224 714 examined using LED-FM, 33 552 (14.9%) were smear-positive, an additional yield of 5251 cases after adjusting for the increase in patients examined. We recommend replacing conventional microscopy with LED-FM in high workload microscopy centres in India.

In 2011, the World Health Organization (WHO) recommended that light-emitting diode fluorescence microscopy (LED-FM) be phased in as an alternative to conventional light microscopy using Ziehl-Neelsen (ZN) stain for the detection of *Mycobacterium tuberculosis* acid-fast bacilli in sputum smears for the diagnosis of pulmonary tuberculosis (PTB).¹ LED-FM using auramine phenol stain has been shown to be more sensitive (~10%) than conventional microscopy in controlled laboratory settings.^{2,3} However, there is limited evidence to show whether routine implementation of LED-FM in the programme setting does indeed improve case detection. In this study, we report on changes in the detection of sputum smear-positive PTB cases before and after the introduction of LED-FM services in the routine programme setting within India's Revised National Tuberculosis Control Programme (RNTCP).

METHODS

Study setting

India has around 350 medical colleges, each of which is attached to a teaching hospital, which usually attracts large numbers of people for health care. To expand access to TB diagnostic and treatment services, the RNTCP has established designated microscopy centres (DMCs) and DOTS centres in almost all the medical colleges. Patients with symptoms suggestive of TB attending these hospitals are referred for smear microscopy per national guidelines. The DMCs are staffed

with trained laboratory technicians and supervised by a senior TB laboratory supervisor, who ensures the quality of sputum smear microscopy in accordance with the RNTCP's quality assurance guidelines.⁴

In early 2012, through a Wave-2 grant from the Stop TB Partnership's TB REACH initiative, conventional microscopy was replaced with LED-FM using auramine phenol staining procedures in 200 medical college DMCs across 23 states countrywide. This involved procurement and provision of LED fluorescence microscopes, training of laboratory technicians and their supervisors (~400) in the use of LED-FM and related quality assurance protocols, which include on-site evaluation, random blinded rechecking of slides and the provision of the necessary laboratory consumables. Procurement, training and implementation, including recording and reporting, followed RNTCP guidelines.⁵ No discordance was observed between the results of the laboratory technician and those of the supervisor during onsite evaluation and random blinded rechecking.

The introduction of the LED-FM services was initiated in May 2012, and by the end of June 2012, 190 of the 200 sites had started using LED-FM. Sputum smear microscopy by ZN staining was discontinued in these sites after the initiation of LED-FM services. Details of all patients examined with smear microscopy for TB and their results were recorded in the RNTCP laboratory registers maintained at each of these centres.

Study population

The study population consisted of individuals with TB symptoms who underwent sputum smear examination by ZN staining during July–December 2011, and by LED-FM during July–December 2012 in 190 medical colleges in India. The same months were chosen in 2011 and 2012 for comparison to minimise the effect of seasonal variation on the number of individuals with TB symptoms examined and smear-positive TB cases diagnosed.^{6,7}

Data source, variables, validation, compilation and analysis

From each DMC, the data on the number of individuals examined for diagnosis using smear microscopy and the number found to be sputum smear-positive for the periods July–December 2011 and July–December 2012 were extracted from the RNTCP laboratory registers.

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KEY WORDS

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The data were validated against the laboratory registers by four technical consultants who were hired under the project and trained for the purpose. Data from all sites were compiled at national level using Microsoft Excel (Palisade Corp, Newfield, NY, USA) and assessed for changes in the number of individuals with TB symptoms who underwent sputum smear examination, smear-positive TB cases detected and the proportion of patients found to be sputum-positive.

Ethics issues

The study protocol was reviewed and approved by the International Union Against Tuberculosis and Lung Disease Ethics Advisory Group. Permission to collect the data was obtained from the Central TB Division of the Ministry of Health and Family Welfare of the Government of India.

RESULTS

The total number of individuals with TB symptoms who underwent diagnostic sputum smear microscopy, the number found to be sputum smear-positive and the proportion of smear-positive patients before and after the introduction of LED-FM is given in the Table. Compared to 2011, in 2012 the number of smear-positive TB cases diagnosed and the proportion of smear-positive patients rose by about 20%, compared to a marginal 1% increase in the number of individuals with TB symptoms examined. Applying 12.6% positivity (observed in 2011) for the additional number of patients examined in 2012, LED-FM services still resulted in an additional yield of 5251 cases.

DISCUSSION

Despite the WHO recommendation to phase in LED-FM for the diagnosis of PTB, little evidence is available with respect to its implementation in high TB burden countries. This is one of the first studies from a high-burden country to show that it is feasible to implement LED-FM services under routine programme conditions in high workload settings, with a significant increase in the number of sputum smear-positive TB cases diagnosed. While we believe that use of LED-FM contributed significantly to enhanced case detection, there could be other possible reasons. First, laboratory technicians and their supervisors at the sites were all retrained in laboratory procedures at reputed national training centres. This could have led to the improved performance of sputum smear microscopy and consequent increase in the number of cases. Second, differences in the demographic and clinical characteristics of the two population groups with TB symptoms being compared (2011 and 2012) may also have contributed to the observed change. We did not collect any additional data to assess these differences, and this is one of the limitations of this study. Furthermore, the LED-FM services were introduced in microscopy

TABLE Performance of LED-FM (July–December 2012) in comparison to conventional microscopy (July–December 2011) in 190 RNTCP designated microscopy centres in India

Period	Total subjects with TB symptoms examined <i>n</i>	Sputum smear-positive TB cases detected <i>n</i>	Proportion found sputum positive %
July–December 2011 (conventional microscopy)	222 658	28 042	12.6
July–December 2012 (LED-FM)	224 714	33 552	14.9
Absolute and incremental change between 2012 and 2011	+2056 (1% increase)	+5 510 (20% increase)	+2.3 (19% increase)

LED-FM = light-emitting diode fluorescent microscopy; RNTCP = Revised National TB Control Programme; TB = tuberculosis.

centres attached to tertiary care teaching hospitals. Whether this can be done at peripheral health facilities needs further assessment. Further research to assess the cost-effectiveness, user-friendliness and acceptability (from the laboratory technician's perspective) of LED-FM is in progress and will be reported in due course.

CONCLUSION AND RECOMMENDATIONS

The introduction of LED-FM services in high workload settings in India was feasible and effective in increasing the detection of smear-positive TB cases. This makes a strong case for scaling up this technology to similar settings in India and elsewhere.

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Dans 190 centres de microscopie des collèges médicaux travaillant dans le Programme Révisé de Lutte contre la Tuberculose en Inde, l'examen microscopique par fluorescence à diode émettrice de lumière (LED-FM) a remplacé l'examen microscopique à lumière conventionnelle utilisant la coloration de Ziehl-Neelsen pour la détection de la tuberculose pulmonaire à frottis positif. Nous avons comparé les performances du LED-FM entre juillet et décembre 2012 à celles de l'examen microscopique conventionnel (juillet–décembre 2011) dans

l'ensemble des 190 sites. Les frottis ont été positifs chez 28 042 (12.6%) des 222 658 patients examinés par microscopie conventionnelle comparé à 33 552 (14.9%) des 224 714 patients examinés par LED-FM, un rendement supplémentaire de 5251 cas après ajustement pour l'augmentation du nombre de patients examinés. Nous recommandons le remplacement de l'examen microscopique conventionnel par le LED-FM en Inde dans les centres de microscopie dont la charge de travail est élevée.

En julio del 2012 la microscopia de fluorescencia con diodos electroluminiscentes (LED-FM) reemplazó el sistema tradicional de microscopia de luz con tinción de Ziehl-Neelsen en la detección de casos de tuberculosis pulmonar con baciloscopia positiva en 190 centros de microscopia de las facultades de medicina que funcionan en conformidad con el Programa Nacional Revisado de Control de la Tuberculosis de la India. En el presente artículo se comparó el rendimiento diagnóstico de este nuevo sistema de microscopia (entre julio y diciembre del 2012) con el rendimiento de la microscopia de luz

(entre julio y diciembre del 2011) en 190 centros. De los 222 658 pacientes investigados mediante la microscopia de luz, 28 042 (12,6 %) presentaron baciloscopia positiva. De los 224 714 pacientes cuyas muestras se examinaron mediante la LED-FM, 33 552 (14,9 %) obtuvieron una baciloscopia positiva, lo cual corresponde a un incremento del rendimiento de 5251 casos, después de corregir con respecto al aumento del número de pacientes examinados. Se recomienda sustituir el sistema tradicional de microscopia de luz por la LED-FM en los centros con un alto volumen de trabajo en la India.