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Original Article

Impact of introducing light-emitting diode fluorescence microscopy services for diagnosis of pulmonary tuberculosis under Revised National Tuberculosis Control Program India

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ABSTRACT

Context: Light-emitting diode fluorescence microscopy (LED-FM) has been recommended by the WHO and the Government of India over the conventional bright-field microscopy using Ziehl–Neelsen (ZN) staining for the diagnosis of sputum smear-positive tuberculosis (TB) suspects. **Aim:** The aim of this study was to assess the effectiveness of LED-FM in detecting TB cases. **Setting and Design:** This retrospective cohort study was carried out in December 2016 using secondary data of the years 2011–2012 from designated microscopy centers (DMCs) at Chandigarh, India. **Methodology:** Two peripheral DMCs where conventional ZN microscopy was used were taken as controls comparable to two peripheral DMCs which used LED-FM services in programmatic settings. The record of all suspected TB patients who underwent sputum smear examination pre- and post-LED-FM introduction was compared for measuring smear-positive cases, conformational grading, and time taken to read per slide examined. Chi-square was applied to assess the statistical significance. **Results:** Out of total 8850 cases registered after the inception of LED-FM microscopy services, case detection rate was 13.3%, with significant decrease in case detection rate at tertiary level DMCs, there was significant increase in case detection rate at peripheral DMCs operating with LED-FM in contrast to ZN microscopy which was used previously. Scanty grade smear increased significantly by 9.0%. The time taken per slide examination decreased by 57.1% (from 7 min on an average to 3 min on an average) after LED-FM was used. **Conclusion:** LED-FM is easy to use, takes lesser time to examine slides, and has longer self-life. It also eases laboratory technician's task in reporting scanty grade positives which use to be challenging previously. The results of this study provide enough evidences to scale up the installation process and usage of LED-FM at DMCs for TB diagnosis in Indian settings.

KEY WORDS: Light-emitting diode fluorescence microscopy, Revised National Tuberculosis Control Program, tuberculosis, Ziehl–Neelsen microscopy

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INTRODUCTION

Tuberculosis (TB) is a major public health threat and is responsible for 1.5 million deaths globally.^[1] As per the WHO Global report, the estimated global TB incidence cases in 2015 were 10.9 million with 25% of them reported

from India.^[1] Globally, India has the highest burden of multidrug-resistant-TB cases and second highest burden of HIV-TB cases. It is estimated that about 170 million workdays are lost annually in India due to this disease

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which is equivalent to an annual economic cost of 130 billion dollars.^[2] Besides, this an undiagnosed TB positive case can infect 10–15 people in his/her entire life.^[3] Such consequences can be catastrophic for our national ambition of eliminating TB and Government of India's pledge of health for all.

Sustainable development goals (2015–2030) and End TB strategy (2017–2025) aims to culminate TB epidemic and reduce the incidence as well as absolute number of TB deaths (baseline 2015) to 90%. Revised National TB Control Program (RNTCP) under its strategic plan for 2012–2017 also envisage for detection of 90% of all incident TB cases as well as to treat them. Among the enlisted strategies of RNTCP, use of sputum microscopy is the primary method of diagnosis for symptomatic cases.^[4] Use of Ziehl–Neelsen (ZN) staining under the direct microscopy was a landmark intervention that increased case detection rate from 40% to 70% in the program settings.^[5] Although ZN staining has high sensitivity and specificity for acid-fast bacilli (AFB), the ambitious goals set by the Government of India to eliminate TB by 2030 needs more technological advancement for diagnosis purposes under RNTCP.^[6]

LED FM has been recommended by the WHO and Government of India to be introduced for diagnostic purposes in place of conventional ZN staining. Studies conducted globally have documented a similar or higher sensitivity (~8%–10%) and specificity for LED FM as compared to ZN staining-based conventional microscopy for sputum smear-positive TB case detection.^[7–12] In addition, LED FM was reported to consume less time per slide examination because slides are examined at lower magnification thus covering much larger area. It is also reported that, as compared to conventional microscopy, LED FM needs lower maintenance and can be operated even on batteries. These features make it suitable for Indian settings where high TB burden is present, and hence, the workload on laboratory staff is excessive.^[9,11] Few infrastructural issues such as irregular electric supply, sluggish equipment maintenance procedures may add on for suboptimal performance which necessitates need of technological advancement-like light-emitting diode fluorescence microscopy (LED-FM) in programmatic settings.^[7,9,11] The RNTCP had a target of replacing the existing conventional microscopy technique by LED-FM with priority at health facilities with higher workload.^[4]

Merely, a solitary study in India by Reza *et al.* in 2013 at 15 randomly selected medical colleges has been conducted which documented that LED-FM has increased effectiveness in diagnosis of TB positives. However, evidence for the same at the peripheral microscopy centers in Indian setting is lacking^[9] which bleaks the advocacy needed for its rapid installation at all the designated microscopy centers (DMCs) in the country.

The present study was done with an overall aim of assessing the effectiveness of LED-FM in detecting

TB cases. Specifically, the objectives are to document difference in case detection rate, to identify any change in conformational grades and to examine time take to read per slide before and after introduction of LED-FM at different levels of healthcare facility.

METHODOLOGY

Study settings

This retrospective cohort study was conducted in Chandigarh, India, with a population of 1.1 million. The organizational structure of RNTCP in India consists of a Central TB division, State TB cell, District TB center, TB unit (TU), DMC, and directly observed treatment short course (DOTS) center. Chandigarh has 3 TU, 17 DMC, and around 171 DOTS centers.^[13] Chandigarh started the revitalization of diagnostic technique in the year 2012 with the introduction of Auramine O based LED-FM at DMCs. Five DMCs were equipped with LED-FM during the period of study, out of which only four were operational.

Each presumptive TB case has to undergo two sputum smear examination with one on spot and another with early morning sputum specimen. A presumptive TB case with one or both sample positives is confirmed as a pulmonary TB case.^[4] LED-FM is now recommended to be used instead of conventional ZN staining microscopy to examine these sputum smears of suspected TB cases.

Study population, sample size, and sampling technique

For the study purpose, four DMCs where LED-FM was operational were selected. Among these, two were in tertiary level medical institutes and two at peripheral health centers. The case detection rates for these DMCs were compared before (2011) and after (2012) introduction of LED FM. In addition, two peripheral DMCs were chosen as controls which were using conventional method (ZN Staining) for the detection of TB cases. These cases and controls were located in same type of localities and cover almost similar populations.

The study population included population with presumptive TB referred to the above-mentioned DMCs for sputum smear examination. The results of sputum examination were compared before (2011) and after the introduction of LED-FM (2012). Assuming 90% of case detection rate, 80% power of the study and confidence interval of 95%, an appropriate sample size of 1200 was calculated.

Study variables and data analytics

The dependent variables were change in case detection rate, AFB grading for cases, and time consumed in examining each slide after the introduction of LED FM whereas, the independent variables were age, gender, types of DMCs, and number of sputum smears examined. These details were collected from the designated RNTCP microscopy laboratory registers. Data were tabulated into the Microsoft Excel and cross-tabulated with pre- and

post-LED-FM introduction. Statistical significance of the result was analyzed by Pearson's Chi-square test, $P \leq 0.05$ was taken statistically significant.

Ethical approval

Ethical and administrative approval for the study was taken from the State TB Program Officer, Chandigarh, to access the record from the DMCs.

RESULTS

A total of 7631 TB symptomatics were examined in the preintervention period, out of which 1049 (13.7%) were found positive. Similarly, 8850 symptomatic were examined postintervention (Introduction of LED-FM) in same setup, out of which 1173 were found positives (13.2%). The patient population characteristics before and after the introduction of LED FM was almost similar [Table 1].

Thus as compared with ZN microscopy, there was a significant decrease in case detection rate after the introduction of LED FM at tertiary DMCs ($P = 0.03$) whereas, a significant increase in case detection rate (percentage change + 13.7%) with LED FM at peripheral DMCs was noticed ($P = 0.04$) [Table 1].

Two similar peripheral DMCs were selected as control where no such intervention was done during the period (2011–2012), and they showed a decrease in case detection rate over the period (percentage change – 7.12%) [Table 2].

There was a significant increase in scanty grading by 9% and 1+ grade by 0.1%, although the 2+ and 3+ grades showed a decrease of 3.1% and 4.2%, respectively, after the introduction of LED-FM for case detection [Table 3].

On observation and interview, it was found that average time taken by laboratory technician (LT) to examine the slide under LED-FM was 3 min (2–4 min) post-LED-FM introduction as compared to an average of 7 min (8–9 min) for ZN microscopy.

DISCUSSION

This study assessed and compared the case detection rate before and after the introduction of LED FM in operational settings. We found that there was a decrease in case detection rate when LED-FM was introduced at tertiary level healthcare institutes. Yet at peripheral health facilities, after the usage of LED-FM, there was a significant increase in case detection rate. Further, the study observed that the number of scanty grade smears increased significantly and time required per slide examination decreased to almost half after using LED-FM at microscopy centres.

Contrary to our finding, study done by Reza *et al.*^[9] Chaidir *et al.*^[10] Xia *et al.*^[11] and Ba *et al.*^[12] found increase in case detection rate after LED-FM usage irrespective of healthcare settings. These studies have credited technical advancement associated with LED-FM for the increase in sensitivity and case detection rate in addition to other factors such as training of staff, better work capacity, and better infrastructure. Similar to our finding, Bonnet *et al.* at healthcare clinic in Kenya,^[14] Minion *et al.*^[15] at tertiary care centers in Canada have documented that there was no rise in case detection rate when LED-FM was used.

Similar to earlier literature,^[9] we found a percentage increase in scanty grade smears ($P < 0.05$) after introducing LED-FM.^[9,11] There are attributes of LED FM which makes it more sensitive than ZN. These are better absorbability of mycolic acid for carbol auramine (used in LED-FM) as compared to carbol fuschin (used in ZN microscopy) resulting in staining larger number of bacilli as well as high-power fields for examining larger field area with easier identification with sharper contrast between background and bacilli.^[16] Moreover, studies have questioned the quality of carbol fuschin powder used for ZN stain.^[8] LTs have showed confidence in reporting scanty grade case after the intervention and this will endorse the RNTCP objective of improving case detection rate.

Table 1: Sociodemographic profile of the patients in the study

Study variable	ZN microscopy		LED FM microscopy		P
	Examined (n)	Positive (%)	Examined (n)	Positive (%)	
Age					
12-44	NA	669	NA	797	
45-75	NA	380	NA	376	
Gender					
Male	4808	698 (14.5)	5931	869 (14.6)	
Female	2823	351 (12.5)	2919	304 (10.4)	
Type of DMCs					
Tertiary center					
PGIMER	2940	353 (12.0)	3772	416 (11.0)	0.03
GMCH-32	3088	453 (14.7)	3164	427 (13.5)	
Peripheral center					
Civil-dispensary	735	106 (14.4)	932	162 (17.4)	0.04
Mani-majra	868	137 (15.8)	982	168 (17.2)	

NA: Data not available, DMCs: Designated Microscopy Centers, LED FM: Light-emitting diode fluorescence microscopy, ZN: Ziehl–Neelsen

Table 2: Comparison of case detection rate between Designated Microscopy Centre before and after introduction of light-emitting diode fluorescence microscopy

DMC type	Year/type	Examined	Positive	Proportion	P
Case	ZN/2011	1603	243	15.1	0.04
	LED FM/2012	1914	330	17.2	
Control	ZN/2011	1069	129	12.1	0.84
	ZN/2012	1035	116	11.2	

DMC: Designated Microscopy Center, LED FM: Light-emitting diode fluorescence microscopy, ZN: Ziehl-Neelsen

Table 3: Sputum smear examination results, by scanty grading in selected Designated Microscopy Centers before and after introduction of light-emitting diode fluorescence microscopy

Grades of the patient	ZN staining, n (%)	LED FM, n (%)	Change (%)
Scanty	211 (20.1)	257 (21.9)	9.0
1+ positives	287 (27.4)	323 (27.5)	0.1
2+ positives	206 (19.6)	223 (19.0)	-3.1
3+ positives	345 (32.9)	370 (31.5)	-4.2

LED FM: Light-emitting diode fluorescence microscopy, ZN: Ziehl-Neelsen

The LTs in our study reported that ZN microscopy took around 7 min (6–8 min) on an average for confirmatory diagnosis whereas LED-FM takes merely 3 min (2–4 min). Moreover, LED-FM working does not require dark room, or warm up time and their diodes work for longer time. Besides it has lesser negative health consequence as no chance of dissemination of mercury vapors as is the case for ZN microscopy.^[15,16] These facts have been documented in earlier literature too.^[8,9] The reduction of time after introducing LED-FM under RNTCP will lessen the laboratory workload which will improve the quality of work. Another Indian study by Bhalla *et al.* has reported a marginal increase in sensitivity with use of LED FM; considerable time saving and convenience.^[17] Contrary, Marzouk *et al.* in Tunisia concluded similar time taken by LED FM as compared to conventional microscopy.^[18]

Strengths

In this study, we covered all the types of healthcare facilities under the domain of RNTCP which provided a comprehensive picture of utility of LED-FM in different settings. This effort was lacking in Indian^[9] as well as global scenarios.^[8,10,11,12] Further, the study covered all the domains suggested under RNTCP for good microscopy such as case detection rate, specificity, time per slide, and ease of working.

Limitations

Since it was a secondary data analysis of aggregated data, we had no check on the data quality, although RNTCP maintains a high standard in data recording procedures. Further nonavailability of patient, wise disaggregated data limited the presentation of results and scope for regression analysis.

CONCLUSION AND RECOMMENDATION

We conclude that there is an increase in scanty sputum cases with a decrease in the time taken by LED microscopy compared to ZN microscopy, which underscores the need for its installation in diagnostic laboratories at all levels of healthcare facilities of developing nations. We suggest future research on disaggregated data for detailed analysis measuring effectiveness of LED FM in diverse healthcare settings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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