

Comparison of fluorescence microscopy and Ziehl-Neelsen technique in diagnosis of tuberculosis in paediatric patients

Zaib-un-Nisa,¹ Humera Javed,² Aizza Zafar,³ Atiqa Qayyum,⁴ Abdur Rehman,⁵ Hasan Ejaz⁶

Abstract

Early diagnosis of tuberculosis is very important for therapeutic reasons and to control the spread of infection. The purpose of this study was to compare the efficacy of fluorescence (FL) microscopy in comparison to Ziehl-Neelsen (ZN) staining. A total of 103 samples were collected from paediatric tuberculosis (TB) suspects and processed using Petroff's method. The smears were subjected to ZN and FL staining for the detection of acid-fast bacilli (AFB). Positive smears were graded according to scale of International Union Against Tuberculosis and Lung Disease and World Health Organisation (IUATLD/WHO). Out of 103 pulmonary and extra-pulmonary samples, 7(6.8%) were positive for AFB on the ZN method, while the positivity increased to 9(8.7%) on the FL method. Two positive samples were missed on ZN staining which were found to be positive with FL microscopy; thus overall positivity increased by 2/9 (22.22%) by FL microscopy over the conventional ZN method. The difference in case detection was found to be statistically significant ($p < 0.00$). FL technique has a better diagnostic value and is less time-consuming compared to ZN in diagnosing tuberculosis in paediatric patients.

Keywords: Fluorescent method, Ziehl-Neelsen stain, AFB, Pulmonary and extra-pulmonary tuberculosis, Paediatric patients.

Introduction

Tuberculosis (TB) is an infectious airborne disease.¹ It is a disease of poverty, affecting mostly young adults. One-third of the world's population is thought to have been infected with *Mycobacterium tuberculosis*.² According to the World Health Organisation (WHO), the incidence of sputum-positive TB cases in Pakistan is 80/100,000 per year. TB is responsible for 5.1% of the total national disease burden in Pakistan.³ Culture of *M. tuberculosis* is the gold standard diagnosis of TB.⁴ However, culture is a slow process requiring specialised laboratories and highly

skilled staff. It takes 6 to 8 weeks before being recorded as negative.⁵ Many automated systems that are faster and accurate include the BACTEC 9000; VersaTREK, mycobacteria growth indicator tube (MGIT), microscopic observation drug susceptibility (MODS) assay and polymerase chain reaction (PCR)-based Gene Xpert mycobacterium tuberculosis/resistance to rifampicin (MTB/RIF) have high operational cost.⁶ Smear microscopy is a simple, economical, less time-consuming technique used for early detection, and it also has prognostic value.⁷ This method is specific, faster and cheaper for the detection of acid-fast bacilli (AFB) in sputum.⁸

Ziehl-Neelsen (ZN) stain is used worldwide as a standard method to detect AFB, but it has less sensitivity relative to fluorescent stain as it takes more time to scan at least 300 fields and often misses the paucibacillary tuberculosis. Fluorescence (FL) microscopy technique has the advantage to examine at lower magnification, allowing the larger area per unit of time.⁹ There is not much data available for the use of ZN and FL microscopy to diagnose the cases of TB in paediatric patients. The present study was planned to compare the role of ZN and light-emitting diode (LED) FL microscopy in the diagnosis of tuberculosis in paediatric patients.

Methods and Results

The descriptive study was conducted from June to December 2012 after ethical approval from the institutional committee. A total of 103 samples were collected from indoor and outdoor paediatric patients suspected of pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB). The patients up to the age of 15 years were included regardless of their gender and the sampling technique was time-dependent. The samples were processed by Petroff's method.¹⁰ The smears were stained using ZN and LED FL staining. Positive smears were graded according to the scale of International Union Against Tuberculosis and Lung Disease and World Health Organisation (IUATLD/WHO).¹¹ FL microscopy was done under 40X, while ZN was 100X.

Of the total, 48(46.6 %) were PTB and 55(53.4 %) were EPTB specimens. The latter included gastric aspirates which accounted for highest proportion 14(25.4%)

^{1,4,5}Department of Pathology, Ghulam Devi Chest Hospital, ^{2,3,6}Department of Microbiology, The Children's Hospital & Institute of Child Health, Lahore, Pakistan.

Correspondence: Hasan Ejaz. Email: hasanmicro@gmail.com

Table-1: Comparative evaluation.

Specimens	ZN +ve	FL +ve	ZN -ve FL +ve	ZN +ve FL -ve	Total AFB +ve	Total number
Sputum	06 (12.5%)	07 (14.6%)	01 (2.1%)	00	07 (14.6 %)	48 (46.60 %)
Extra-Pulmonary	01 (1.82%)	02 (3.64%)	01 (1.82%)	00	02 (3.64%)	55 (53.40 %)
Total	07 (6.8 %)	09 (8.74%)	02 (1.94%)	00	09(1.94%)	103

$\chi^2 = 1.970, p < 0.00$

ZN: Ziehl-Neelsen

FL: Fluorescence.

Table-2: FL and ZN Staining (n=103).

Results	FL	ZN
No AFB seen	94 (91.26%)	96 (93.20%)
Scanty	01 (0.97%)	04 (3.9%)
1+	05 (4.85%)	02 (1.94%)
2+	02 (1.94%)	0 (0%)
3+	01 (0.97%)	01 (0.97%)

FL: Fluorescence

ZN: Ziehl-Neelsen

AFB: Acid-fast bacilli.

followed by pleural fluid 13(23.63%), pus 10(18.18%), pericardial fluids 5(9.09%), joint fluid 5(9.09%), ascitic fluid 4(7.27%), cerebrospinal fluid (CSF) 2(3.64%), tracheal aspiration and bone marrow 1(1.81%) each.

Out of 48 PTB samples 6(12.5%) were positive by ZN stain and 7(14.6%) by FL staining. Among 55 EPTB samples, 1(1.8%) was positive by ZN stain and 2(3.64%) by FL microscopy. There were 7(6.8%) AFB smear-positive samples on the ZN method while the positivity increased to 9(8.7%) on the FL method. Two positive samples were missed on ZN staining which were found to be positive with FL microscopy. Overall positivity thus increased by 2/9 (22.22%) using LEDFL microscopy over the conventional ZN method. The difference in case detection was found to be statistically significant ($p < 0.00$) (Table-1). FL microscopy had more positive predictive value (PPV) than ZN microscopy. Comparison of 103 cases by both microscopic techniques was done separately (Table-2).

Discussion

The detection of AFB is often considered evidence of the infected state. In developing countries, microscopy of the specimen is the fastest, cheapest and the most reliable method for the detection of AFB. Since early 1940s, the comparison of the FL method with the conventional ZN method on sputum smears was implemented to improve smear-positivity for the detection of AFB. In our study, 9 (8.7%) samples were positive by FL stain and 7(6.8%) were

positive by ZN stain. Out of 48 pulmonary samples 6(12.5%) were positive by ZN stain and 7(14.6%) were detected by FL staining. Out of 55 EPTB samples, 1(1.8%) was positive by ZN stain and 2(3.64%) by FL microscopy. These results showed that FL staining technique is more sensitive in the detection of AFB in PTB as well as EPTB samples compared to ZN stain. The result is in accordance with a study that found positivity for AFB by ZN method 44.11% (45/102) while the positivity increased to 81% (83/102) by FL method.¹² The variability in PPV in contrast to the present study is because our study included EPTB and PTB samples only from paediatric patients that have low positivity rates. According to the present study, a total of 9 samples were positive for AFB. FL microscopy detected 2/9 paucibacillary samples that were missed on ZN staining; thus positivity increased by 22.22% by FL microscopy. In Jaipur, India, FL detected 9.29% paucibacillary samples that were missed on ZN staining and these results are close to our results.⁷

Conclusion

FL technique has a better diagnostic value compared to ZN stain in paediatric patients where the case detection rates of FL over ZN were comparable to those found by several studies. FL microscopy was less time-consuming compared to ZN method. FL method improved diagnostic value, especially in patients with a low density of bacilli. LED FL microscopy reduced the expense and there is no need for a separate dark room for microscopy.

References

1. Cheesbrough M. District laboratory practice in tropical countries Part-2. UK: Cambridge University Press, 2005; pp 207.
2. World Health Organization. Global Plan to Stop TB. 2011-2015. (Online) (Cited 2012 July 22). Available from URL: <http://www.who.int/mediacentre/factsheets/fs104/en/index.html>.
3. National TB Control Programme. Burden of TB. (Online) (Cited 2012 July 23). Available from URL: <http://www.ntp.gov.pk/about.htm>.
4. Kumar V, Abbas AK, Fausto N, Mitchell RN. Robbins Basic Pathology. 8th ed. Philadelphia: Saunders Elsevier; 2007.
5. Levinson W. Review of Medical Microbiology and Immunology. 11th ed. New York: McGraw Hill; 2010.
6. Drobniowski F, Caws M, Gibson A, Young D. Modern laboratory

- diagnosis of tuberculosis. *Lancet Infect Dis* 2003; 3: 141-7.
7. Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas, L. Comparison of Ziehl-Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. *Indian J Tuberculosis* 2011; 58: 72-6.
 8. Khatun Z, Hossain M S, Roy CK, Sultana T, Rahman MQ, Samad A, et al. Light Emitting Diode (LED) Fluorescent Microscopy: a Milestone in the Detection of Paucibacillary Mycobacterium in Case of Pulmonary Tuberculosis 2011. *Bangladesh Med J* 2011; 40: 22-6.
 9. Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. *Kathmandu Uni Med J* 2009; 7: 226-30.
 10. Acid-fast bacillus smear and culture. (Online) 2012 (Cited 2012 July 24). Available from URL: <http://labtestsonline.org/understanding/analytes/afb-culture>.
 11. NTCP. Manual for Sputum Smear Fluorescence Microscopy Revised. (Online) 2007 (Cited 2012 July 24). Available from URL: <http://tbcindia.nic.in/pdfs/Flourescence-Microscopy%20Manual.pdf/>.
 12. Annam V, Kulkarni MH, Puranik RB. Comparison of the modified fluorescent method and conventional Ziehl-Neelsen method in the detection of acid fast bacilli in lymphnodeaspitates. *Cytojournal* 2009; 6: 13
-