ORIGINAL ARTICLE

COMPARISON OF ZIEHL–NEELSEN BASED LIGHT MICROSCOPY WITH LED FLUORESCENT MICROSCOPY FOR TUBERCULOSIS DIAGNOSIS: AN INSIGHT FROM A LIMITED RESOURCE-HIGH BURDEN SETTING

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Background: Microscopy is the most widely used tool for Tuberculosis screening. Conventionally, Ziehl-Neelsen (ZN) staining has been the widely used for staining Acid-Fast Bacilli (AFB) but with the advent of Fluorescent staining, Auramine O stain is now being adapted as the preferred method for setups with high workload as it has the advantage of being less laborious, since bacteria fluoresce in front of a dark background and are easier to count. This study was performed to compare the efficiency of the two methods in a high-burden, limited resource setting to see the magnitude of diagnostic accuracy between ZN and Fluorescent Microscopy, using culture as the standard. **Methods:** Altogether 987 culturally confirmed cases were considered from the period 36 months during January 2011 to December 2013 and data were compiled from the records maintained at the Provincial Tuberculosis Reference Laboratory at Ojha Institute of Chest Diseases, Dow University of Health Sciences, Karachi. The results from 523 cases examined using ZN and 464 cases using Fluorescent staining method were compared for diagnostic accuracy on the basis of Mycobacterial culture results. Smears are prepared from the clinical samples obtained from presumptive tuberculosis patients. **Results:** The results of ZN method showed 94.23% [95% CI 91.32–96.39%] sensitivity and 84.91% [95% CI 78.38–90.08%] specificity. While FM showed a sensitivity of 97.15% [95% CI 94.82–98.63%] and specificity of 83.19% [95% CI 74.99–89.56%]. **Conclusions:** The results showed that Fluorescent microscopy was slightly more sensitive than ZN light Microscopy, while specificity of both the methods were comparable.

Keywords: *Mycobacterium tuberculosis*, Acid fast bacilli, Tuberculosis, Ziehl-Neelsen (ZN) staining, Auramine O Fluorescent staining

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INTRODUCTION

Tuberculosis is a major global health problem. It affects millions of poor people alone and in combination of HIV and is the leading cause of death and disability worldwide.[1] Early diagnosis of tuberculosis is important for therapeutic reasons and to control the spread of the infection.[2]

Most of the world's Tuberculosis cases occur in low income countries, and the diagnosis and treatment monitoring depends upon the use of labour intensive, easy to use methods with minimum infrastructure, which makes microscopy the most widely used tool for Tuberculosis screening.[3]

The causative agent, *Mycobacterium tuberculosis* is an acid-fast bacillus and cannot be stained by ordinary Gram staining methods because of high lipid content in its cell wall. These lipids called Mycolic acids make the organism resistant to decolorization by acid alcohols, which gives them the name acid-fast.[4]

Smear microscopy is a simple, economical, less time-consuming technique used for the early detection of tuberculosis.[5] The purpose of microscopy is to detect acid fast bacilli in clinical specimens. Both viable and non-viable bacilli are stained and get counted. The results of examination of stained smears are reported in a standardized way so that results can be compared. The commonly used scoring systems are published by World Health Organization (WHO), International Union Against Tuberculosis and Lung Diseases (IUATLD) and American Centre for Disease Control (CDC).[6]

Ziehl Neelsen (ZN) staining is the conventional method for the screening of Tuberculosis using light microscope, however, it is gradually being replaced by fluorescent staining which uses Auramine O stain to visualize bacteria easily.

Currently used Fluorescent microscopes are expensive, as they make use of specifically designed Light-emitting diodes (LED) for fluorescence microscopy rather than conventional methods. The carbol fuchsin and Auramine O used in these techniques each function by binding to Mycolic acids in the mycobacterial cell wall. Fluorescent stained bacteria are bright yellow against a dark background

allowing the slides to be scanned under low magnification without losing sensitivity.[7]

LED based fluorescent microscopy offers qualitative, operational and cost advantages over both the conventional fluorescence and Ziehl-Neelsen microscopy.[8]

MATERIAL AND METHODS

A total of 987 culturally confirmed diagnostic cases were considered from the period 36 months during January 2011 to December 2013 and data were compiled from the records maintained at the Provincial Tuberculosis Reference Laboratory at Ojha Institute of Chest Diseases, Dow University of Health Sciences, Karachi.

Among these cases ZN based microscopy was performed on 523 cases and LED based fluorescent microscopy was performed on 464 cases by the same microscopists. The results of microscopy were compared with the culture results as a standard.

The smears were prepared using WHO guidelines and stained using ZN and Fluorescent staining methods and microscopically graded according to the World Health Organization protocols. ZN smears were examined under 1000X and Fluorescent smears were examined under 400X magnification as per guidelines.[9] Positive and negative controls were also set up for every batch as a routine procedure.[10]

RESULTS

Out of 464 specimens processed by using Auramine O Fluorescent stain, 360 were positive. Among these 341 were true-positives and showed growth of AFB on culture medium, the smear results were compared with the solid culture that was used as a standard, LED Fluorescent microscopy showed a sensitivity of 97.15% [95%CI 94.82–98.63%] and specificity of 83.19% [95% CI 74.99–89.56%], PPV of 94.72% and NPV of 90.38%. Out of 523 samples that used for evaluating the efficacy of ZN staining and microscopy 367 samples were positive while 343 were also positive on culture medium. ZN light microscopy has a 94.23% [95%CI 91.32–96.39%] sensitivity and 84.91% [95% CI 78.38–90.08%], PPV of 93.46% and NPV of 86.54%. The results are summarized in table-1 and table-2 and shown graphically in figure-1.

Data was recorded and tabulated using Microsoft Excel. Statistical analyses were performed using Online statistical Medcalc Software.[11]

DISCUSSION

Our results did not show a significant numerical advantage of Fluorescence Microscopy on ZN as the sensitivity, specificity, PPV and NPV were all comparable. This was in line with a number of studies which compared ZN versus conventional and LED fluorescent microscopy and came to the similar conclusion[12,13] though some have described some diagnostic advantage of fluorescent over ZN microscopy[3,14]. However, it has been explicitly observed and reported by multiple studies that the time spent on fluorescent microscopy is half that of ZN, which increases the efficiency of the process and is especially helpful in high burden settings. Fluorescent Auramine O staining procedure is also simple as compared to ZN staining method does not require heating.

Using fluorescent microscopy, increased rate of smear positivity was noticed. This may be due to the fact that Auramine staining offers more contrast, the bacilli appear as brilliant yellow against a dark background, thus making it easier for the reader to pick up even low number of bacilli.[3]

Our results did not show a significant advantage of fluorescent microscopy over traditional method, but in high burden settings, its use saves time and increases efficiency. The fluorescence microscopy provides rapid screening of smear specimens. The laboratories where large numbers of smears are examined per day and more time is consumed on confirming the negative smear results, the rapid screening of smears become highly advantageous. According to the International Union against Tuberculosis and Lung Disease technical guidelines for sputum microscopy, for the correct identification of negative smear 5 minutes screening time is required by using the conventional light microscope. While fluorescent microscopy needs 1 min. to examine a smear.

Ba *et al.* reported even better timings and showed that the mean time required for fluorescence microscopists to declare a slide as negative using the same magnification was 3 minutes 34 seconds, which when compared to the light microscopy was 7 minutes 44 seconds using ZN technique.[13] Our results support the evidence that LED Fluorescent microscopy can be used as an effective alternative to ZN conventional method as it facilitates the improvement of diagnostic services

CONCLUSION

Our results support the evidence that LED Fluorescent microscopy can be used as an effective alternative to ZN conventional method as it facilitates the improvement of diagnostic services. Though it needs slightly more expertise, and training, it can be a good alternative to ZN based microscopy, specially in high burden settings.

AUTHORS' CONTRIBUTION

MYN: Conceived the, performed the analysis, wrote part of the draft, supervised the project. ZAli and FA: Performed the lab work. SS: Conceived the idea, partly wrote the draft.

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REFERENCES

1. WHO. Global tuberculosis report 2015. [Internet]. [cited 2016 May 1]. Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf
2. Zaib-un-Nisa, Javed H, Zafar A, Qayyum A, Rehman A, Ejaz H. Comparison of fluorescence microscopy and Ziehl-Neelsen technique in diagnosis of tuberculosis in paediatric patients. J Pak Med Assoc 2015;65(8):879–81.
3. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, *et al*. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006;6(9):570–81.
4. Collins CH, Grange JM, Yates M. Tuberculosis bacteriology: organization and practice. Butterworth Heinemann; 1997.
5. 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 Tuberc 2011;58(2):72–6.
6. Global Laboratory Initiative. Mycobacteriology laboratory manual. Geneva WHO Stop TB Partnersh. 2014.
7. Winn WC, Koneman EW, editors. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
8. FIND. Partnering for better diagnosis for all. Foundation for Innovative Diagnostics. [Internet]. [cited 2015 may 1]. Available from: https://www.finddx.org/wp-content/uploads/2016/04/delivering-on-the-promise-Sep2008.pdf
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 Univ Med J (KUMJ) 2009;7(27):226–30.
10. Salam AA, Rehman S, Munir MK, Iqbal R, Saeed SS, Khan SU. Importance of Ziehl-Neelsen Smear and Culture on Lowenstein Jensen Medium in Diagnosis of Pulmonary Tuberculosis. Pak J Chest Med 2014;20(2).
11. MedCalc. Statistical Software, Update 2014. [Internet]. [cited 2015 May 1]. Available from: www.medcalc.org
12. Minion J, Pai M, Ramsay A, Menzies D, Greenaway C. Comparison of LED and conventional fluorescence microscopy for detection of acid fast bacilli in a low-incidence setting. PLoS One 2011;6(7):e22495.
13. Ba F, Rieder HL. A comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis 1999;3(12):1101–5.
14. Trusov A, Bumgarner R, Valijev R, Chestnova R, Talevski S, Vragoterova C, *et al*. Comparison of Lumin LED fluorescent attachment, fluorescent microscopy and Ziehl-Neelsen for AFB diagnosis. Int J Tuberc Lung Dis 2009;13(7):836–41.

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