Original Research Article

Comparison of laboratory diagnostic methods for diagnosis of pulmonary tuberculosis

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Abstract

Background: Tuberculosis is an important global public health problem. Early diagnosis of tuberculosis is essential to ensure early identification of cases and good treatment outcomes. In developing countries like India, microscopy of the specimen is by far the fastest, cheapest, and a reliable method. **Aim:** This study was aimed at comparing Ziehl-Neelsen staining and Auramine O Fluorescent staining of sputum samples taking culture on Lowenstein-Jensen medium, as "gold standard" for the diagnosis of pulmonary tuberculosis. **Material and Method:** Total 210 suspected pulmonary tuberculosis patients were included in this cross sectional study. Two sputum samples were taken from each patient and subjected to ZN staining, Auramine O fluorescent staining and culture on LJ medium. **Result:** Out of 210 cases 34 (16.19%) were positive on culture. Smear positivity rate of ZN stain was 12.86% and fluorescent stain was 15.23%. The concordance between ZN and fluorescent staining was 97.62%. Agreement between ZN staining and culture was 95.71% while agreement between Fluorescent staining and culture was found to be 98.1%. The sensitivity, specificity, positive predictive value and negative predictive value of ZN staining were 76.47%, 99.4%, 96.29% and 95.45% respectively. The sensitivity, specificity, PPV and NPV of fluorescent staining were 91.17%, 99.4%, 96.88% and 98.25% respectively. **Conclusion:** Auramine O fluorescent staining is more sensitive than ZN staining for the diagnosis of pulmonary tuberculosis.

Key words: tuberculosis, ZN staining, Fluorescent staining.

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INTRODUCTION

Tuberculosis (TB) is an important global public health threat and it is also one of the world's leading causes of death due to an infectious agent. It is estimated that between years 2009-2025 nearly one billion people will be newly infected, 200 million people will be affected with TB and out of it 40 million are likely to die from it if control programs are not improved.[1] Around twenty six percent of global annual TB incidents occur in India which makes it a country with highest tuberculosis burden in the world. [2] Early diagnosis of TB is essential to ensure proper and early identification of cases and good treatment outcomes in order to limit its transmission. [3] Lack of rapid and effective methods for diagnosis of TB is a major problem faced by the developing countries. [4] The diagnostic methods for TB should be cost effective, rapid, reliable and easy to perform. As per International standards for tuberculosis care, microscopy, culture and molecular methods are the major diagnostic modalities available today.[4] In developing countries like India, microscopy of the specimen is by far the fastest, cheapest, and a reliable method. Under Revised National Tuberculosis Control Programme (RNTCP), sputum smear microscopy is the

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mainstay of the diagnosis of pulmonary tuberculosis. Most commonly used staining methods are Ziehl-Neelsen staining and fluorescent staining with Auramine-O/Auramine - Rhodamine using light-emitting diode (LED) fluorescent microscope. [5] The tubercle bacilli can be demonstrated microscopically by both ZN stain and fluorescent stain. Though both methods have same basic principle, but there is difference in sensitivity of these two methods.[6,7,8] Considering all the above information, in the present study the ZN staining and Auramine O Fluorescent staining of sputum samples were compared and evaluated taking culture on Lowenstein-Jensen medium, as "gold standard" for the diagnosis of pulmonary tuberculosis.

MATERIAL AND METHODS

This cross-sectional study was carried out after obtaining the Institutional Ethics Committee approval. Study was conducted in Microbiology department of a tertiary care teaching hospital from November 2016 to October 2018. Total 210 patients referred to microbiology department with clinical suspicion of pulmonary tuberculosis having symptoms like cough with or without expectoration for >2 weeks, fever, weight loss, fatigue, night sweat, haemoptysis, loss of appetite and/or radiological evidence of tuberculosis were included in the study.

Statistical analysis:

Descriptive analysis was done and expressed in terms of mean and percentages. Sensitivity, specificity, positive predictive value and negative predictive value of ZN staining and fluorescent staining were calculated. Chi square test was used to analyze the data. $p<0.05$ was considered as statistically significant.

Specimen collection: two sputum specimens (one spot-specimen A and one early morning- specimen B) were collected from each patient.

Microbiological Work-up

Smear was prepared from purulent portion of the sputum.

Staining: Fluorescent staining (using Auramine O stain) and ZN staining were performed and reported as per the RNTCP guidelines. All positive smears were graded as per RNTCP guidelines.[9]

Culture: After preparation of smears, the remaining sputum specimen was digested and decontaminated by Kudoh swab method (10) and was inoculated on LJ medium.

RESULTS

Results of ZN staining and fluorescent staining

Out of 420 specimens, 54 (12.86%) were positive by ZN staining and 64 (15.23%) were positive by fluorescent staining. Smear positivity rate of ZN stain was 12.86% and fluorescent stain was 15.23%.

Concordance between ZN and Fluorescent staining

410/420 (97.62%) smears showed concordant results and10/420(2.38%) smears showed discordant results. 10 smears were positive by fluorescent staining but negative by ZN staining.

Culture positivity

Out of 210 cases 34 (16.19%) were positive on culture. Therefore the overall frequency of new pulmonary tuberculosis cases was 34 (16.19%) in the study population.

Gender wise distribution of culture positive pulmonary tuberculosis cases

Out of 34 culture positive cases, 24 (70.59%) were males and 10 (29.41%) were females with Male: Female ratio of 2.4:1.

Age wise distribution of culture positive pulmonary tuberculosis cases

Maximum positivity was observed in the age group of 21-40 years (64.71%) followed by the age group 41-60 years (17.64%). Statistically, age-specific prevalence was found to be significant ($X2 = 11.42$, $p = 0.009$).

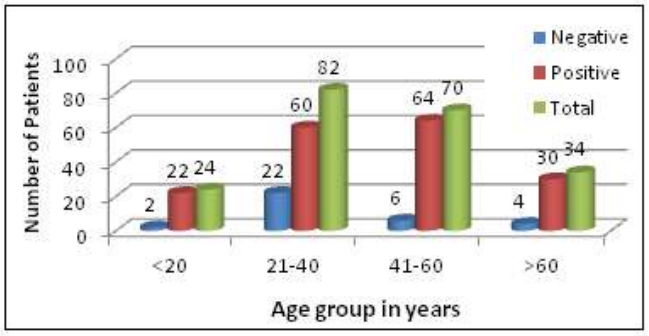

Chart: Agewise distribution of culture positive case

Comparison of microscopy and culture findings

Of the total 68 culture positive specimens, 52 (76.47%) were positive on ZN staining while 62 (91.17%) were positive with fluorescent staining. Agreement between ZN staining and culture was 95.71% while agreement between Fluorescent staining and culture was 98.1%. Grade wise correlation of ZN staining and fluorescent staining is shown in Table 1.

Table 1: Grade wise correlation of ZN staining and fluorescent staining

	POSITIVE BY	
GRADING	ZN Staining	LED FM
Scanty	8 (14.81%)	16(25%)
1+	10(18.52%)	12(18.75%)
2+	14(25.93%)	14(21.88%)
3+	22(40.74%)	22(34.37%)
Total	54 (100%)	64(100%)

The sensitivity, specificity, PPV (Positive Predictive Value) and NPV (Negative Predictive Value) for the

diagnosis of Pulmonary tuberculosis was calculated for ZN smear microscopy and fluorescent smear microscopy by taking culture on LJ medium as gold standard. Samples that were positive and negative in culture were considered true positive and true negative respectively. Culture negative and ZN or fluorescent microscopy positive samples were taken as false positive samples. Culture positive and microscopy negative samples were taken as false negative.

Table 2: Evaluation of ZN and fluorescent microscopy against culture

Sr. No.	Measures	ZN vs Culture (%)	LED FM vs Culture (%)
1	Sensitivity	76.47	91.17
2	Specificity	99.4	99.4
3	PPV	96.29	96.88
4	NPV	95.45	98.25

DISCUSSION

Smear positivity by ZN and fluorescent staining

In current study smear positivity rate of ZN stain was 12.86% and fluorescent stain was 15.23%. Our results are in agreement with studies by Mamilla R. *et al*[11], Vijaya D. *et al*[12], Bhumbla U. *et al* [13], Golia S *et al* [14], Kumar J. *et al*[15] and Marais B. *et al*[16]. In the present study, smears were made from direct sputum specimen without using any concentration method. Higher positivity rate in other studies may be correlated with concentration method used.

Concordance between ZN staining and fluorescent staining

Present study revealed 97.62% concordance between ZN and fluorescent staining. We found more concordance than some other studies. Laifangbam S. *et al*[17] had reported 70.6% agreement between ZN and fluorescent staining while in the study conducted by Goyal R. *et al* [18] the concordance between two methods was 92.7%. Bhumbla U. *et al* [13], and Ziaee M. *et al*[19] showed the concordance of 92.3% and 74.5% between these two staining methods respectively.

Total culture positivity

In the present study, 34/210 (16.19%) cases were culture positive with overall frequency of new pulmonary tuberculosis cases being 16.19%. Marais B *et al*[16] and Kumar JS. *et al* [15] reported similar finding with culture positivity of 16% and 19.5% respectively. Getachew *et al* [20] have reported culture positivity rate of 13.5% in HIV-positive patients. Higher culture positivity was reported by Malhotra B. *et al*[21] (48.5%), Laifangbam S. *et al* [17] (70.6%) and Rao VG *et al* [22] (81.9%). In present study Kudoh method was used for decontamination of sputum specimen. Other methods of digestion and decontamination were used in other studies by different workers which have resulted in higher culture positivity rate.

Gender wise distribution of pulmonary tuberculosis cases

Male predominance was noted among the pulmonary tuberculosis cases in the present study. Similar findings were noted by other workers like Vijayalakshmi T.*et al* [23] (M:F ratio -2.3:1), Myneedu *et al* (24) (M:F ratio -1.7:1) and Alemayehu M. *et al* [25] (M:F ratio -1.7:1). Rao VG *et al* [22] from India reported M:F ratio of 3.3:1 in culture positive cases. Getachew *et al* [20] have reported culture proven TB prevalence in 62.5% males and 37.5% females among HIV-positive patients. As per the WHO, global male:female ratio for tuberculosis notifications in 2017 was found to be 1.7:1.[26] There are reports stating that estradiol enhances the immunity by augmenting macrophage activation whereas as testosterone acts as an immune response inhibiting mediator.[27] Sharma PP *et al* [28] have stated that males are having higher risk factors like smoking, alcoholism and drug addiction to acquire tuberculosis than females. Horton KC *et al* [29] suggested that lesser number of females among the pulmonary tuberculosis cases may be due to the barriers faced by females in seeking healthcare and getting diagnosed with TB.

Age wise distribution in pulmonary tuberculosis cases

In the present study, maximum pulmonary tuberculosis cases belonged to the age group of 21-40 yrs which comprises of working population. Age association in our study was found to be statistically significant. Our results are in concordance with other authors such as Gaur PS *et al* (30) (20-40 years), Malhotra B *et al* (23) (35.46+ 16.91 years) and Alemayehu M. *et al* (25) (30-40 years). Rao VG *et al* (22) reported highest pulmonary tuberculosis in age group of >65 years.

Comparison of ZN staining and fluorescent staining with culture

Sensitivity and specificity of direct ZN staining reported by various authors have been found in between the range of 51% to 94.23%; and 84.91% to 100% respectively; whereas sensitivity and specificity of direct fluorescent staining reports were between 57% to 97.22%; and 83.19% to 100% respectively. The lowest sensitivity of ZN staining (29.2%) was reported by Getachew *et al* [20] in direct sputum samples of HIV-positive patients. In the present study, the sensitivity of Fluorescent staining method is significantly higher than that of ZN staining method. This is in accordance with various studies by Laifangbam S. *et al* [17], Ulukanligil M. *et al* [31], Ziaee M. *et al* [19] and Agrawal M *et al* [32]. In the present study, ZN staining and fluorescent staining methods have equal specificity. Ulukanligil M. *et al* [31], Cattamanchi A. *et al* [8] and Ziaee M. *et al* [19] have also reported equal specificity

of ZN and fluorescent staining methods. Even in HIV-positive patients, the specificity of both ZN staining and fluorescent staining was 100% as reported by Getachew *et al*. [20] Noori *et al* [33] have reported the PPV and NPV of ZN staining and fluorescent as 93.46% and 86.54%, and 94.72% and 90.38% respectively. In the study by Laifangbam S. *et al* [17] ZN staining was found to have PPV and NPV of 95.55% and 49.12% while fluorescent staining had PPV and NPV of 95.89% and 93.1% respectively. Ziaee M. *et al* [19] reported the PPV and NPV of ZN staining and fluorescent staining as 100% and 94%, and 100% and 95% respectively. These studies show that PPV of ZN and fluorescent staining are almost equal while NPV of LED fluorescent staining is more than that of ZN staining. These findings correlate well with the present study. The study clearly indicates that the case detection rate (efficacy) of fluorescent microscopy is higher than that of ZN light microscopy. There is higher (98.1%) agreement between fluorescent staining and culture than between ZN staining and culture (95.71%). The use of fluorescent staining alone can be a reliable microscopic method for diagnosis of pulmonary tuberculosis as there were no ZN staining positive cases that were fluorescent staining negative.

Grade wise distribution of smears

In the present study out of 10 smears which were fluorescent staining positive but ZN negative, eight were of scanty grade and two were of grade 1+. These findings indicate that fluorescent staining method helps in increased detection of paucibacillary cases. The bright appearance of AFB against the dark background creates a good contrast in fluorescence microscopy so that scanty smears are also detected. Golia S *et al* [14] proved that fluorescent staining was more efficient over ZN staining in determining paucibacillary pulmonary tuberculosis cases. Khatun Z *et al* [34] reported that only 4% cases of grade scanty were detected by ZN whereas LED fluorescent microscopy detected 14.67% cases. Thus Khatun Z *et al* [34] declared that fluorescent microscopy is a better method than ZN staining due to its close comparability to the gold standard culture technique. Studies by Golia S *et al* [14] and Khatun *et al* [34] noted that more paucibacillary cases were detected by fluorescent microscopy as compared to ZN light microscopy. Therefore, Auramine O fluorescent staining increases the diagnostic value of the sputum smear, especially in paucibacillary specimens that are likely to be missed on ZN staining.

CONCLUSION

Amongst the ZN and fluorescent staining method fluorescent staining is more sensitive and equally specific and is especially useful in detection of paucibacillary cases.

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